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FILM FORMATION AND STRUCTURE OF SOME OIL EMULSIONS [1]

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INTRODUCTION

Incident to the development of certain oil emulsion sprays designed primarily to reduce water losses from leaves, stems, and fruits, studies have been made of certain characteristics of the films formed by different emulsions. This article reports observations on variations in film structure, as influenced by (1) certain changes in the composition of the emulsion, (2) changes in method of application, and (3) differences in the character of the surface to which they are applied.

MATERIALS

The spray materials used are divided, somewhat arbitrarily, into three groups.

Group 1.—Oil-in-water emulsions, represented by a series developed by the section of agricultural chemistry of the Michigan Experiment Station for the purpose of reducing the rate of water loss (transpiration) from plant foliage. These emulsions were prepared from plant oils of quick drying properties, and include various mixtures of linseed, soybean, and castor oils. In addition, small amounts of ammonium salts of fatty acids and proteins, together with bentonite, form the emulsifying systems.

Group 2.—Commercial oil sprays which are used as insecticides. These consist chiefly of more or less refined petroleum oils dispersed in water. The emulsifiers are for the most part trade secrets. The patents of the Standard Oil Company declare as emulsifiers for "Dendrol" a rosin or fatty acid soap (U. S. 1,785,451); for "Stanolind" glyceryl naphthenate (U. S. 1,949,799); and for "Superla" an oil-soluble hydroxy ester of organic acids (U. S. 1,949,798).

Group 3.—Certain miscellaneous materials.

METHODS

PREPARATION OF FILMS

White glass, extra thin, microscope slides were used as surfaces for the formation of films. They were thoroughly cleaned, kept in absolute alcohol, and then dried and polished before being used.

The spray materials were applied to the slides in different ways in order to determine the effect of the method of application on the

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structure of the film. The materials were applied with (1) an ordinary hand atomizer; (2) a high-pressure spray pump; and (3) by placing a drop of the emulsion on a slide and spreading it evenly and thinly by drawing another slide over it. No essential difference in structure of films was observed as a result of the different methods of application.

Under both (1) and (2) the vertical exposure of slides to the spray, as compared with their horizontal exposure, was tried. This produced a difference which, while not fundamental, should be mentioned. The downward flow of the spray material upon application to the slides held in vertical position resulted in very thin, more or less imperfect films on the upper part of the slides and thicker, more or less distorted films, with some peculiarities of structure, over the lower part. This effect will be discussed and illustrated later. Heavier coatings of the spray materials were obtained by successive applications to the same slides after their former coatings had dried and become solid films. Applications of the different materials were made upon slides which had been previously coated with different film-forming substances: gelatin, isobutyl-methacrylate diluted in xylol, agar-agar (3 percent), and paraffin (melting point 40° C.).

In one series of preparations the lower sides of the slides were coated over and blackened with india ink. The aim was to reduce, if not eliminate, the reflection of light from the glass.

Only freshly prepared materials were used, and applications were made within 1 or 2 hours after their preparation. The different emulsions were always thoroughly agitated before they were applied.

The dry oil films formed by the different methods of application, except on vertically exposed slides, were uniform over the contact surfaces, firm in their cohesive and adhesive properties, and definite and observable in their microscopic structural features, with respect to all the materials of group 1 (oil-in-water emulsions, prepared in the Michigan station chemical laboratory). Some of the materials of group 2 (commercial oil sprays) used as insecticides formed the same type of film as those in group 1, while others formed a different type of film, which is illustrated and discussed later in this report.

The slide preparations were stored in slide cases at room temperatures. Kept in this manner, they remained unaltered for considerable periods of time, at least as long as 8 months.

STAINING OF FILMS

Two methods for use with the materials of group 1 were devised and found satisfactory. In the first, the oil emulsion was in a concentrated form, requiring a dilution with two parts of water before its application. The water used for dilution was colored with 0.2 percent Nile blue and then filtered before its addition to the emulsion. After application, and upon drying, the oil phase remained colorless and the aqueous phase blue. All attempts at counterstaining the dried films were unsatisfactory. In the second method, the two phases of the emulsion were stained separately before being mixed: the oil with 0.15 percent scarlet red, the aqueous system with 1 percent Tieman's blue. Mixing the two in the required proportion gave a reddish-blue emulsion, which, upon being applied and allowed to dry, retained the colors of its component parts—red oil globules, blue continuous phase.

In regard to the materials of group 2, which were obtainable only in prepared form, staining of the dried films on the slides was made possible by the use of 1 percent aqueous Nile blue solution. The excess of stain was removed by washing in distilled water. This left the continuous phase blue, the dispersed phase colorless. Attempts with counterstaining, e. g., with scarlet red in 70-percent alcoholic solution, served only to increase the difficulty of clear differentiation between the two phases.

EXAMINATION OF FILMS

Observations were for the most part limited to two principal features: (1) The course of film formation during the process of drying, and (2) the film's structure after it had dried and become fixed. Various microscopic techniques were used in these studies.

The instrument used was a Zeiss binocular microscope, with magnifications arranged at 120 ×, 300 ×, and 600 ×. The source of light was a 50-watt filament bulb, placed behind a blue glass screen. Certain variations in the direction of illumination, size of aperture, length of focus, and so forth, were employed as necessary. Furthermore, all the preparations were also examined with the aid of "Ultrapak" equipment, in order to secure vertical illumination, with an arc light as its source.

The limitations of the microscope for such studies are recognized. It does not bring under observation the elemental units and the arrangement of such units in the basic structure of emulsion films. For this purpose, resort must be made to such measurements as surface tension and potential and to employment of the ultramicroscope. However, despite their limitations, microscopic examinations of emulsion films yield important information about them.

Bütschli (*4*),[3] from microscopic observations of colloidal materials, reported a "honeycomb" or "alveolar" structure for them, and assumed this type of structure to be characteristic of both living and nonliving matter. Copisarow (*5*) observed "periodical" structures with colloids, and also "honeycombs" with dehydrated emulsions. Friedel (*6*) describes several different types of structural forms of emulsions: rods, polygonal areas, conic and nuclear configurations. He assumed that this diversity of form depended upon temperature and the degree of dilution of the materials he studied.

When emulsions are properly applied to receptive surfaces and allowed to dry thereon, their more elemental units coalesce into larger units (aggregates), and these aggregates further unite into more or less orderly and characteristic configurations. Then these configurations are microscopically observable and describable.

By studying the film structure in terms of these configurations it is possible to obtain information on the orientation and distribution of the coarser structural elements as well as on the coarser structures of the films. The final result from efforts to observe and comprehend the behavior of these aggregated particles may be, as some workers contend, a true and useful way to arrive at an understanding of the elemental and invisible film structure.

A Leitz Panphot Universal Camera was used to make the microphotographs of the prepared slides. Magnifications obtained by

[3] Italic numbers in parentheses refer to Literature Cited, p. 251.

means of this equipment were about 500× and 1,000×. Approximately 200 microphotographs were made of the slides at hand. Reproductions showing typical conditions are presented in the following section.

RESULTS

MATERIALS OF GROUP 1 (OIL-IN-WATER EMULSIONS)

The Formation of Films

When emulsions of oil in water, which include many spray materials, are spread over a receptive surface and allowed to dry, the final result is a solid, fixed film. Various physical (evaporation of free water) and chemical (oxidation, polymerization, etc.) phenomena are involved and interwoven in the drying process.

The transformation from liquid to solid is not instantaneous. Time intervenes during which the progress of the change, which results in the formation of larger and larger particles and their aggregation into permanent configurations, may be observed under the microscope.

Figures 1 and 2 illustrate the general film-forming behavior of oil-in-water emulsions. They show successive stages in the drying process from the application of the emulsion (7.5 percent oil, 1 percent bentonite) to the slide, up to the final completely dried and permanent film.

Caught in figure 1, *A* (emulsion immediately after application) is a curving streamlike motion of the water which carries the dispersed oil globules passively along with it. Variation in size of the globules was from the limit of visibility to 16 microns in diameter. The smallest ones (diameters up to 4 microns) showed Brownian movement. The larger globules are relatively few in number. They not only vary in size but also in transparency, from being wholly transparent to considerably clouded. Adherence of the globules, especially of smaller ones to form larger ones, is conspicuous. This indicates a force of attraction which is high, since the interfacial tension between like molecules of oil is low (*1*, *2*). It presages the coalescence of globules to form globular units of larger size, which have the smallest possible surface areas for the volumes concerned. Other globules may be kept from contact with each other by their electric charges and mutual repulsion.

Figure 1, B, shows the same coating after the lapse of 1 minute. Streaming continues, but is more curvilinear in direction. The smaller globules are still being carried along in the current, but the larger ones, both singly and grouped, have come to rest. The number of visible globules has increased. Possibly not all of these are composed of oil alone. According to Adam (*1*) they may represent the various combinations of the phases of the emulsion, in this case, oil, protein, bentonite. Their emergence out of the complex continuous phase is perhaps mostly marginal, since at the edges of the film the globules behave like molecules when the surface is contracting. That is, they continuously move inwardly because of not being balanced in their inward-moving tendency by outward-moving globules of the same number (*1*).

The phenomenon of "multiple emulsion" (*13*) is apparent in several of the larger globules. This consists in a globule of the dispersed phase being an emulsion by containing, within itself, smaller globules

of the other phase, or phases. Adam (*1*) has suggested that this occurs only when at least two different emulsifying agents are present.

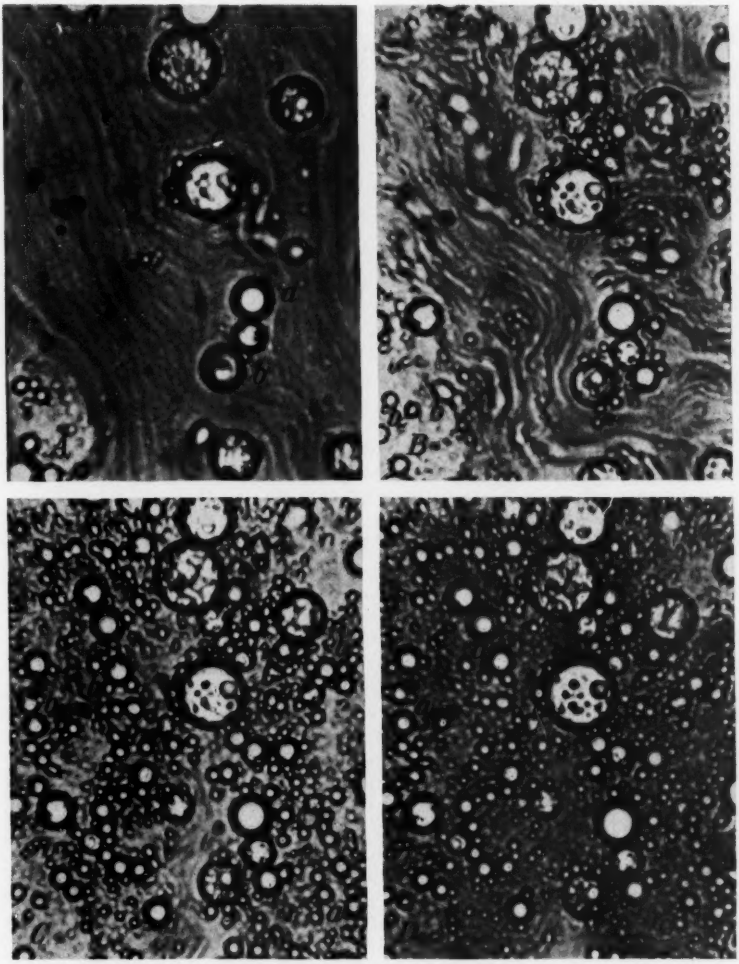

FIGURE 1.—Drying stages of a spray film of an emulsion of 7.5 percent oil, 1 percent bentonite. *A*, Film in the fresh state immediately after application on glass. Arrows, Streaming motion; *a*, transparent oil globule; *b*, clouded globule; *c*, multiple emulsion. *B*, Film after 1 minute; largest oil globules have come to rest: *a*, Multiple emulsion; *b*, coalescence. *C*, Film after 3 minutes: *a*, Coalescence of small globules; *b*, attraction of globules. *D*, Film after 5 minutes; streaming motion has practically ceased: *a*, Repulsion of one globule by another. All ×1,000.

This was substantiated in the present study by the observation that "multiple emulsion" occurred only with the materials of group 1, in which the emulsifying system contained bentonite in addition to fatty

acids and proteinates. The tendency for newly emerged globules to cluster together and the coalescence of some globules are shown as being under way.

Further developments are shown in figure 1, *C* which illustrates the film after the lapse of 3 minutes. The solution is still motile, but only slightly so. Definite streaming is confined mostly to a narrow channel in the center of the lower half of the figure. In, or out of this moving area small globules are still coming into visibility. The number of globules is considerably greater than that shown in fig. 1, *B*. Many which are only vaguely visible and others not as yet visible in figure 1, *B*, have appeared definitely. The phenomena of attraction between globules, and of their coalescence, are more abundantly illustrated in figure 1, *C*, than in *B*.

Figure 1, *D*, shows the film after 5 minutes of drying. Perceptible motion has virtually ceased. It continues, however, to some degree in the two or three lighter (less dry) patches which are readliy distinguishable.

Attention is directed to what appear to be cases of globular repulsion. This is evident from a comparison of *C* and *D*, for the groups of globules concerned in the action. This tendency exists and is operative, in contrast to the stronger and more general movement towards adherence and the ultimate coalescence of the dispersed globules.

Loss of motility is a function of the rate of drying, and this rate is more or less contingent upon the temperature, the particular composition and concentration of the emulsion, and the thickness of the film. For the materials of group 1, under the varied conditions of the observations made, the minimum time for loss of motility was 5 minutes, with an occasional maximum of 10 minutes.

During the 5 to 10 minutes immediately following the cessation of perceptible motility, certain changes take place. A description of these changes, as observed and illustrated in figure 2, is briefly as follows: Very small gaps were seen to open in the stationary globules. These openings appeared suddenly, taking no longer than a so-called "split second." The gaps closed as rapidly as they opened, and left behind no marks of their occurence. A hypothetical explanation of this phenomenon could be that of an excessively high capillary pressure exerted by entrapped air bubbles, directed towards the centers of the globules. This was antecedent to a depression of the spherical walls of the globules and led to the immediate disappearance of the air bubbles. Watching this, one might think that these bubbles were being swallowed by the surrounding fluid. Actually, however, they may have been bursting, as a consequence of increased surface pressure which was due to the compression of the film by increased drying.

Subsequently, the coalescence of the smaller globules, on a rather general scale, was observed. The larger oil globules became almost instantaneously deformed and showed radial protrusions which broke up their strictly spherical shapes (fig. 2, *A*). Presumably, at least, this is a manifestation of an increased pull on these globules because of augmented surface tension due to increased evaporation of water from the film. Upon the completion of this stage, the spherical oil globules have changed into areas with outlines which are more or less angular (hexagonal tendency) and the consequence is the typical "honeycomb" structure shown in figure 2, *B*, *a*, as described by Bütschli (*4*).

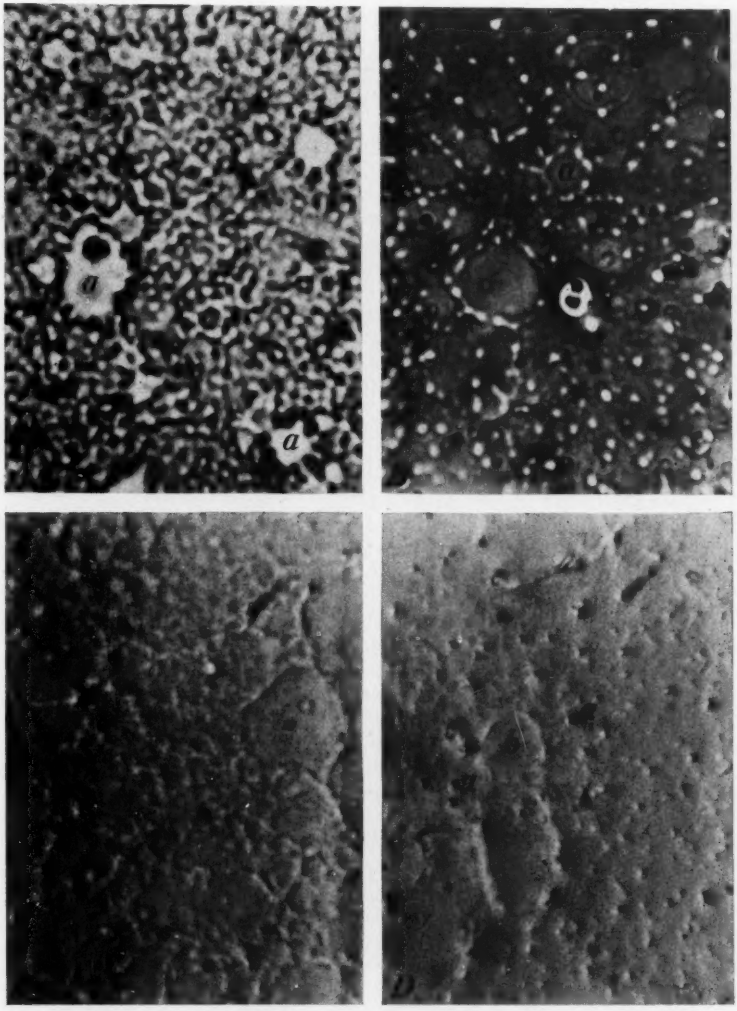

FIGURE 2.—Progressive film formation, oil-in-water emulsion. *A*, Film after 8 minutes of drying: *a*. Amoeboid form of a drying oil globule. *B*, Latest stage in formation of solid film; film after about 10 minutes of drying: *a*. "honeycomb" area; *b*, oil globule not yet dried. *C*, Film completely dried, under ordinary focus. *D*, Film completely dried, under high focus: *a*, elevated nodules; *b*, crateriform depression. All × 1,000.

The explanation Seifriz (*14, p. 197*) gave for the formation of hexagons of drying mud may also explain the emulsion systems under consideration:

> . . . When a body is in a state of strain, as is true of drying mud, the lines of force set up are at maximum stability when three emanate from a point so as to form three angles of 120°. It is for this reason that hexagons are formed when mud dries. . . . The tendency of liquids and semifluid (plastic) systems to adjust themselves to or toward this condition, accounts for the prevalence of the hexagon . . . angle.

It will be noted in figure 2, *B*, that the apparently blank, hexagonal areas are outlined and somewhat connected by chains of brighter spots which appear to be the remaining aggregates of crowded particles from dried globules. These chains form sheaths around the blank more or less hexagonal areas. If their bright spots are not tightly packed together as in a string of beads, but some distance remains between them, they are attached to each other by rodlike bridges. These spots might be assumed to be mere optical artifacts. That they are not such is indicated by the observation that at and above all magnifications of 600 × they passed in all directions through the film, under both transmitted and incident light, with all variations of focus and of size of aperture.

Figure 2, *C*, shows a completely dried and permanently fixed film, under ordinary focus. The honeycomb appearance of structure persists. The light, circular spots, seen serving as borders and connecting lines for the larger blank areas in *B* as such have mostly disappeared. The rims of surrounded areas and the lines connecting them now appear to be solid, with a rough, nodular, uneven aspect.

Figure 2, *D*, shows a completely dried film when observed under high microscopic focus. Two structural elements are most conspicuous: pimplelike, elevated nodules and crateriform, polyhedrous depressions whose bordering rims are characterized by elevated, linked-together nodules. Thus, the final pattern appears to be that of a kind of mosaic, with the residual, polyhedrous figures of the dispersed oil globules outlined, and connected more or less completely, by the dried aggregates of the emulsifying agents. These same agents, in a dried state, constitute the basic, continuous, "warp and woof," so to speak, of the solid film coat.

Variation in the Composition of the Emulsion

The process of film formation so far described and illustrated is based upon observations on a single one (7.5 percent oil, 1 percent bentonite) of the several materials of group 1. This representation is fundamentally typical for the group. However, certain superficial alterations, worthy of attention, occurred when the proportional amounts of the constituents of the emulsion were varied.

In general, the effect from proportionally less oil and more of one of the solid components in the emulsion (3 percent oil, 3 percent bentonite) was, as shown by figure 3, *A*, that of a finer network and a denser film structure. With proportionally more oil and less of the solid constituent (10 percent oil, 3 percent bentonite), the result, as shown by figure 3, *B*, was that of a film less dense in character. The hexagonal areas are more numerous and larger in size, and the interstices between them are more of the nature of peripheric seams, or chain linkages.

Superimposed Films

Repeated applications of the same material were made to previously applied and dried films. The results are illustrated in figure

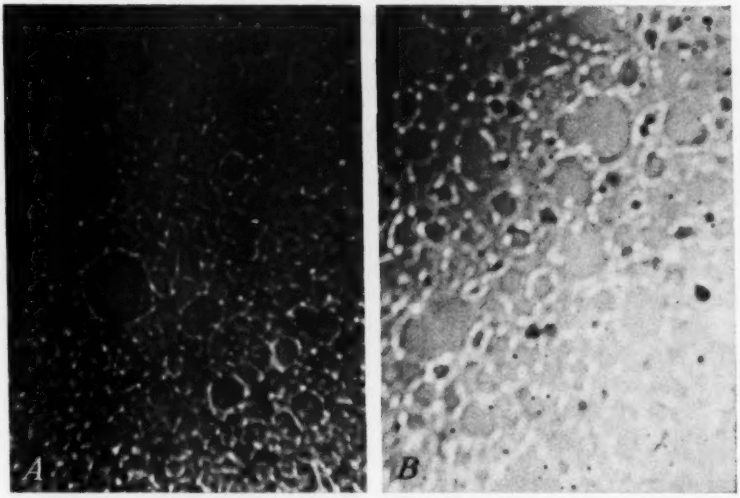

Figure 3.—Variation in the composition of oil-in-water emulsion. *A*, Emulsion film of 3 percent oil, 3 percent bentonite, 1 coat. *B*, Emulsion film of 10 percent oil, 3 percent bentonite, 1 coat. × 1,000.

4. *A* and *B* of this figure show effects secured by two and four applications of an emulsion composed of 3 percent oil and 3 percent bentonite.

Progressive thickening and densification of the film is obvious. Distortion and destruction of the original pattern is equally obvious. Adherence between the successive films to form a final film with greater thickness was perfect, except for a peculiarity which is noted in figure 4, *B*. Dark spots appeared here and there in the film. These might be interpreted as the effect of air bubbles entrapped between the successive layers and not discharged in the process of attaining final equilibrium between these layers.

Figure 3, *B*, shows, in conjunction with figure 4, *C*, *D*, a series of one, two, and four coats of an emulsion composed of 10 percent oil and 3 percent bentonite. Clearly, the effect of successive coats in terms of densification of the film and distortion of its pattern is less pronounced. Furthermore, the presence and survival of encircled dark areas, such as shown in figure 4, *C*, was always observed.

The Effect of Different Substrata

The wetting properties of certain spray materials on different types of plant surfaces and on paraffin-coated slides has been observed by O'Kane and his coworkers (*7*, *8*, *9*, *10*, *11*). Their studies showed clearly that the physicochemical properties of both the spray ma-

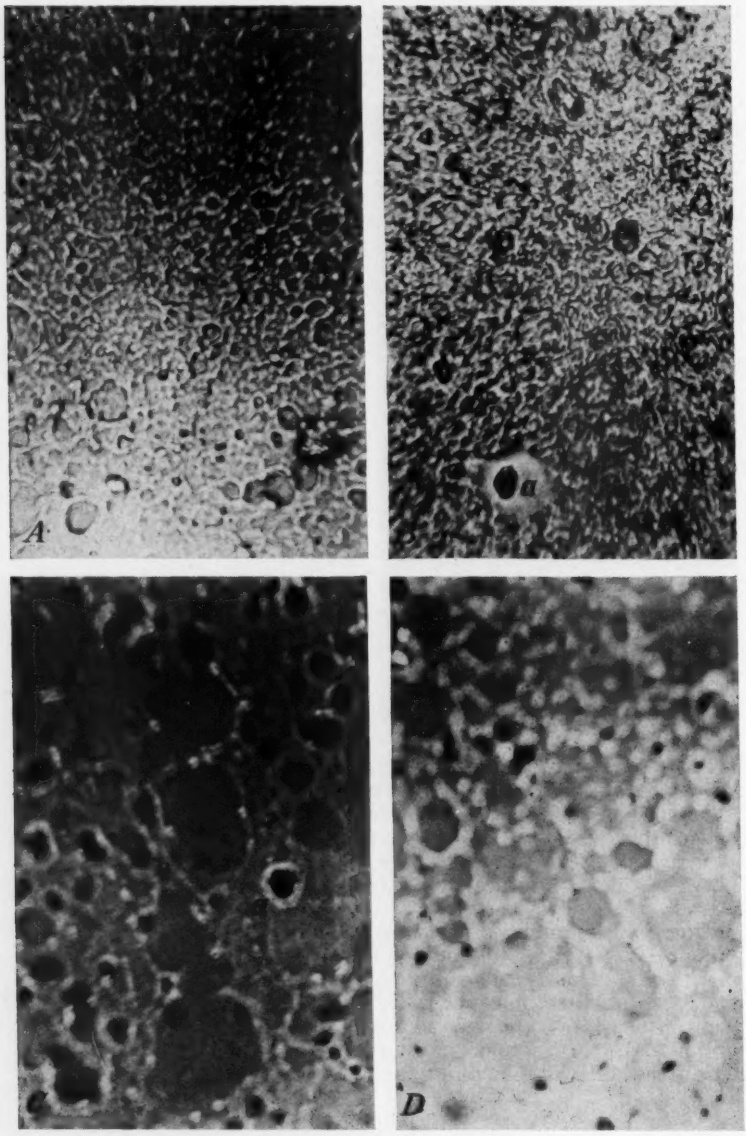

FIGURE 4.—Superimposed films, oil-in-water emulsions. *A*, Two coats of an emulsion of 3 percent oil, 3 percent bentonite. *B*, Four coats of an emulsion of 3 percent oil, 3 percent bentonite; *a*, presumably an air bubble. *C*, Two coats of an emulsion of 10 percent oil, 3 percent bentonite; *a*, oily spaces of both layers superimposed. *D*, Slide coated four times with emulsion of 10 percent oil, 3 percent bentonite. All × 900.

terials and the substrata to which they were applied need to be considered if an understanding of the formation and the visible structure of spray films is to be obtained. The molecular movement, in the liquid state, of an emulsion (spray material) may determine the nature of the final orientation. On the other hand, the fixed molecular orientation of the solid surface, or substratum, with its existing surface energy influences the liquid's adhesive possibility and its tendency for orientation during the process of its drying and becoming permanently filmlike in character.

Different substances were used for coatings on glass slides in order to secure a variety of substrata for the application of one of the oil-in-water emulsions—7.5 percent oil, 1 percent bentonite. These substances included gelatin, isobutyl-methacrylate, agar, and paraffin. Basic films (surfaces) of each of these four substances were produced by spreading the substance over the slide in a thin layer and allowing it to dry and solidify thereon, at room temperature.

With the exception of paraffin, continuous, transparent, microscopically structureless films were obtained. Paraffin is not, as the others are, an optically isotropic substance; a paraffin film has a distinctly patterned structure. This properly places it among the materials of group 3. It will be illustrated and discussed in a later section.

Figure 5, *A*, shows a dried film of the emulsion on a surface of gelatin; *B*, a film of the same emulsion on a surface of isobutyl-methacrylate, and *C*, the same emulsion on a surface of agar. These films may be compared with those in figure 2, *B–D* (dried films of the same emulsion on plain glass surfaces). The three films in figure 5 differ among themselves, and none is identical in aspect with those on the glass surface (fig. 2, *B–D*). However, alterations due to the substrata are only superficial; the fundamental honeycomb structure persists and prevails, and shows merely changes in the proportional relationships of its basic features.

In figure 5, *A* (gelatin surface) the honeycomb structure is notably irregular and dense. The hexagonal areas are numerous, mostly small in size, crowded together to the point of overlapping, and are in general neither sharp in outline nor clear in their contents. Their intermixture with the emulsifying agents is pronounced. Evidently certain areas concentrate more of the liquid than others, and strands of compact lines are formed. This possibly is indicative of contact angles of larger sizes, which, in turn, are antecedent to decreased spreading potentiality of the emulsion.

On a surface of isobutyl-methacrylate (fig. 5, *B*) the openlike spaces of the film are especially distinct and large in size. They are relatively few in number and separated in their locations. The framework of meshes, consequently, composes the larger part of the entire film, and has quite distinctly spotted chains for its connecting strands. Apparently the substratum has good wettability. A high tensile stress, operating at and inwardly from the periphery of the film seems to have expanded the oil globules, while the aggregates of the rest of the material become localized and concentrated in the matrix of the film.

The agar surface (fig. 5, *C*) seems to have exercised the most pronounced influence on the spread and expansion of the emulsion on the film. The adhesive force of the solid agar surface for the

emulsion appears greatly to exceed the cohesive forces of the ingredients of the emulsion within itself. Consequently, the open

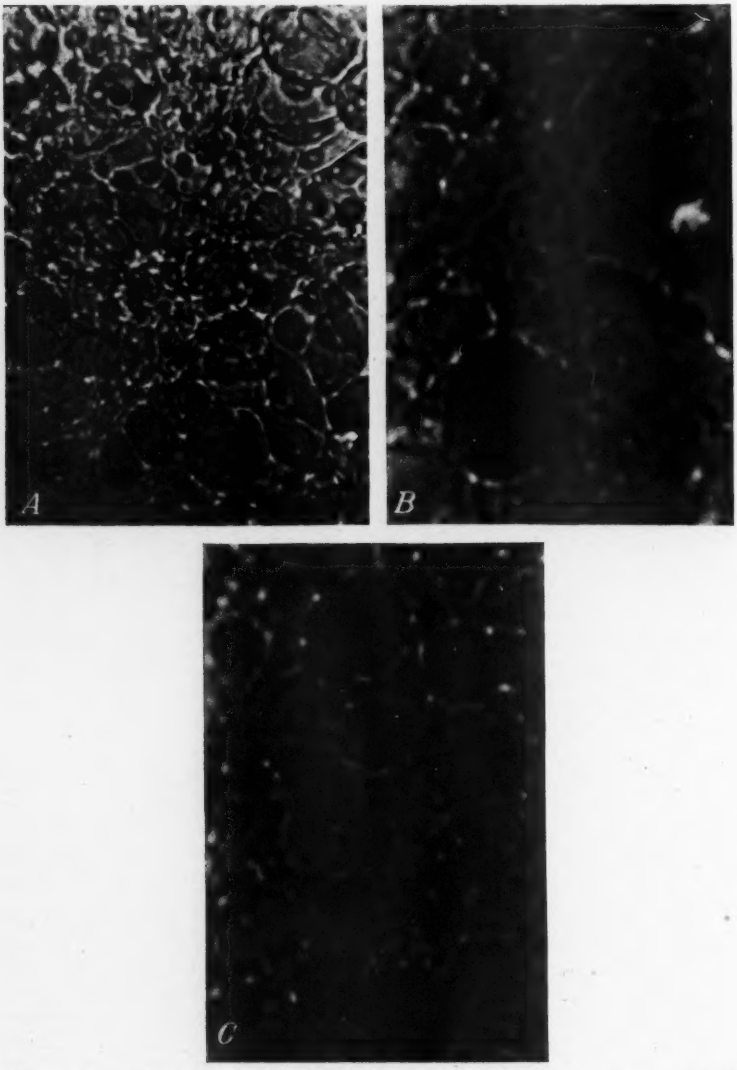

FIGURE 5.—Effect of different substrata with oil-in-water emulsions. *A*, Dried film of oil-in-water emulsion (7.5 percent oil, 1 percent bentonite) on gelatin surface. *B*, Dried film of same emulsion on a surface of isobutyl-methacrylate. *C*, Dried film of same emulsion on agar surface. × 1,000.

spaces (dried oil globules) are relatively large, numerous, close together and almost coherent, and separated only by their respective

rims, the rest of the system being minor in area and spongelike in appearance.

In general, then, the emulsion films superposed on these three types of surface varied in detail, with respect to the relative distribution of the materials of the emulsion, but showed the same fundamental honeycomb pattern that they exhibit when produced on a plain glass surface. They apparently responded individually to the decisive factor of the proportion between the perpendicular and the lateral forces of adhesion which are operative in film formation on different types of surfaces.

MATERIALS OF GROUP 2 (COMMERCIAL OIL SPRAYS)

The materials of group 2, the commercial petroleum-oil sprays, included both dormant- and summer-spray oils. The former contain higher concentrations of oils than the latter. As a group, these emulsions differ from the materials of group 1 principally in that they contain somewhat less effective emulsifying and stabilizing components. Hence, although possessing sufficient spreading and wetting ability for ordinary use as sprays, they are relatively unstable in comparison with the materials in group 1. This lack of stability causes them, when applied, to spread rapidly and to break readily. Far from being considered detrimental to their efficiency as insecticides, this property of quick spreading and breaking is said to be advantageous (U. S. Patent No. 1,949,799). The results from trials with six of these commercial materials are illustrated in figures 6 to 9.

Figure 6, *A*, shows a prepared film of "Standard Aphid Spray Oil," in 2-percent concentration with water, on a glass slide surface. Shiny, circularly outlined spots appear which are irregularly distributed throughout an opaque isotropic matrix. In some places the bright globules are in close proximity and then appear to be arranged in short chains like strings of beads. In such places the film appeared to be somewhat thicker than elsewhere.

Figure 6, *B*, shows a film of "Stanolind," 4-percent concentration, on a glass surface. The bright spots are more numerous than in the film formed by a 2-percent concentration of Standard Aphid Spray Oil (fig. 6, *A*), and they are more generally intermixed and crowded together. They give some evidence of a faintly defined structure that is reticulate in character.

Figure 6, *C*, shows a film of "Dendrol," 4 percent concentration. This film differs from those shown in *A* and *B* principally in the greater number and smaller size of its spots and the fact that they are all mostly transparent. Close scrutiny reveals that these spots tend to form chains of from 2 to 10 units and that these chains tend somewhat towards the formation of a netlike pattern.

Figure 6, *D*, shows a film formed by a 1-percent concentration of "Superla." This spray yields a coarse emulsion, in a reverting state (passing from the oil-in-water to a water-in-oil system). The interfacial tension is thereby reduced to a minimum. The aqueous phase shows no tendency to form spherical droplets, but appears in large, irregular-shaped drops. Some of these contain small oil globules, indicating the presence of a "bimultiple" system (*13*). The dark outlines of the water spaces are the optical consequence of different refractive indices between the two zones. The inner line is lighter, which indicates that the external phase has the higher index.

Figure 7 shows films of the material known as "Free-Mulsion," 4-percent concentration. *A* shows the lacelike pattern of the film which formed on the lower portion of a vertically suspended slide.

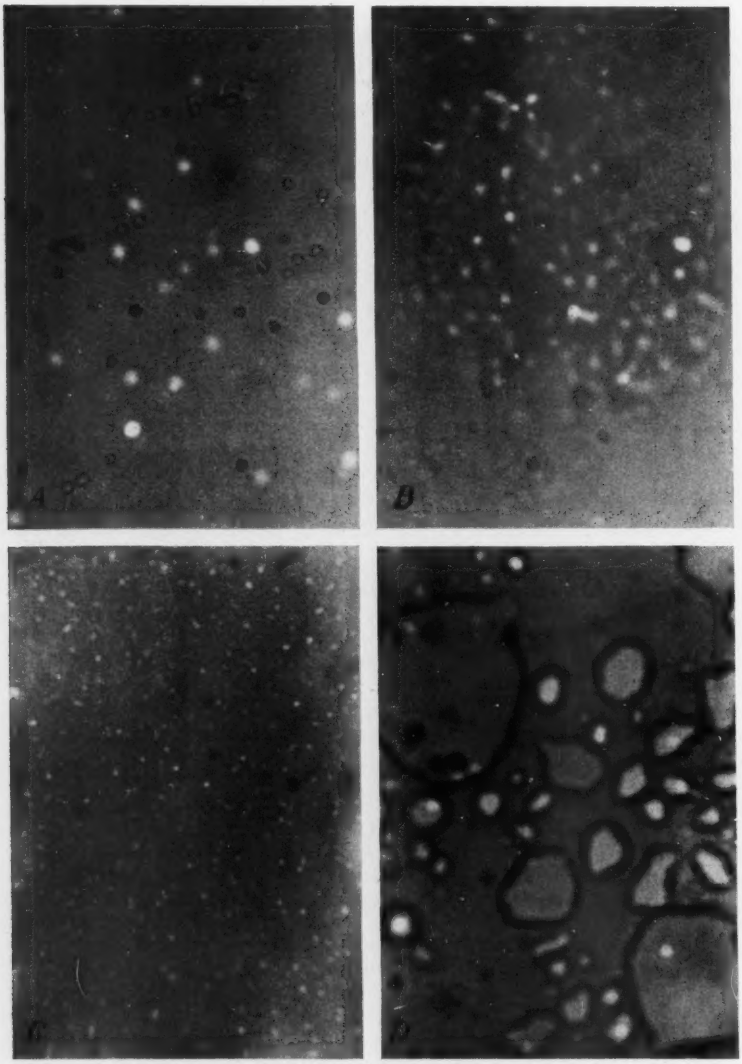

FIGURE 6.—*A*, Film of Standard Aphid Spray Oil on glass. × 1,000. *B*, Film of Stanolind on glass. × 1,000. *C*, Film of Dendrol on glass. × 450. *D*, Film of Superla on glass. × 450.

The emulsion is in an initial state of demulsification. Its surface tension is greater than that of the previously described material

Superla, but the adhesion between the liquid and the glass was too weak to give an even film. The upper part of the film (not illustrated) was thinner, with only a few small and scattered globular areas in its composition. The larger defined areas shown in *A* were formed

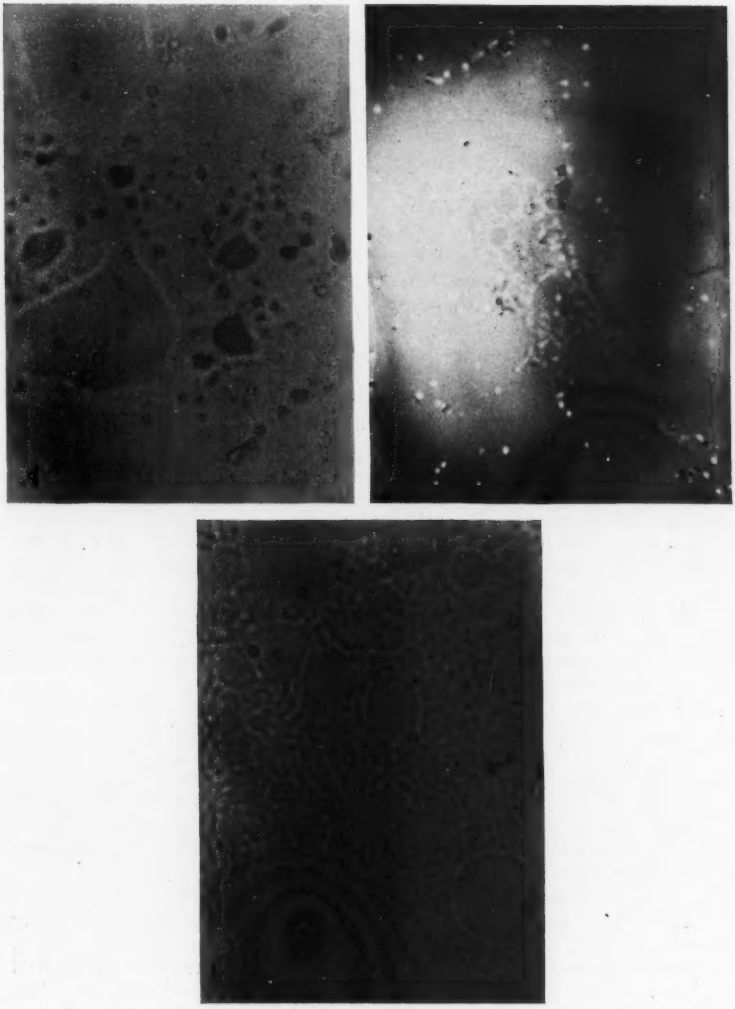

FIGURE 7.—*A*, Film of Free-Mulsion on vertically exposed glass slide. × 1,000. *B*, Film of Free-Mulsion on horizontally exposed glass slide. × 450. *C*, Film of Free-Mulsion on gelatin-coated, vertically exposed slide. × 1,000.

by droplets of oil assembling in pools, coalescing, and losing their separate identities.

Figure 7, *B*, shows a film of the same material, which was formed on a slide horizontally exposed to the spray. A difference due to the

position of the receptive surface, with this quick-breaking emulsion is noteworthy. There appears to be an aggregation of particles, with these aggregates alined in the form of filaments, which link together to encircle the globular areas and so produce an elongated, netlike pattern. In the areas outside of the patterned ones appear only scattered spots with little or no contact. There were certain channels of flow while the material was in the fluid state, and these elongated patterns tended to develop in and out of these currents of liquid motion. Especially at the border of the streaming fluid the aggregates are deposited densely enough to become oriented into filaments.

Free-mulsion was applied also to slides bearing a gelatin film on their surfaces. The results are shown in figure 7, *C*, when the exposure of the surface to the spray was vertical. The contrast with *A* (also vertical exposure) is interesting. Obviously, the adhesive force between the gelatin surface and the emulsion is strong. The contact angles apparently are small and decisive. Despite the vertical exposure of the surface and the down pull of gravity for the freshly applied material, the coating remains almost uniformly thick over the entire surface. Furthermore, it comes to have a continuous structure that is similar to the honeycomb pattern formed by the oil-in-water emulsions of group 1.

Figures 8 and 9 show the results obtained with a material called Summer-Mulsion, 1-percent concentration.

Figure 8, *A*, represents a film of this emulsion on a vertically exposed slide. In the wake of the downflow of the solution there appears a mass of small globular spots interspersed through the solid matrix. Some degree of order appears in the partial union, and even coalescence, of the globules into groups, and also into chains. Figure 8, *B*, shows a film of this material on a horizontally exposed slide. This film contrasts strikingly with that in *A* (vertical exposure of slide). Dark, nodular demarcation lines enclose circular and polyhedrous areas. These vary in size and are joined together by more or less loosely connected aggregates. The dark (black) spots may be interpreted as air pores. Figures 8, *C*, shows another place in the same film as that pictured in figure 8, *B*, but the two images are very different. That in *C* shows much similarity to the lacelike pattern produced by Free-Mulsion (fig. 7, *A*). This quality of pattern in the same film may have resulted from a condition of nonuniformity of the emulsion itself with respect to density, dispersion, or some other property. The place where the lacelike pattern appears indicates the behavior of an unstable emulsion, on the point of reverting (*12*).

Figure 9, *A*, shows the film produced by an application of Summer-Mulsion, 1-percent concentration, to a gelatin-coated slide, vertically exposed. Aside from a relatively few outlined, somewhat larger, circular areas, the aspect is that of a continuous, indefinitely figured netting, composed of dark-centered, lightly outlined spaces. The aggregated particles are mostly in direct contact with each other.

Figure 9, *B*, shows the result of applying Summer-Mulsion, 1-percent concentration, to a surface of isobutyl-methacrylate on a glass slide. The image, obtained by illumination from below, shows medallionlike forms, scattered here and there throughout the entire film.

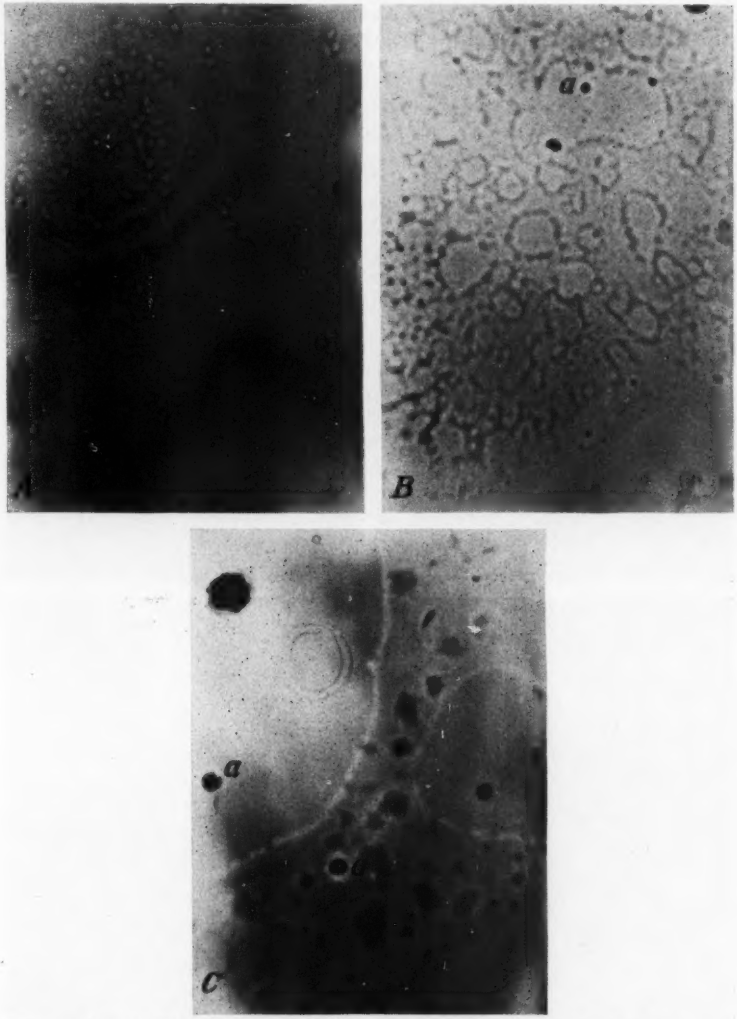

FIGURE 8.—*A*, Film of Summer-Mulsion on vertically exposed glass slide. *B*, Summer-Mulsion on horizontally exposed glass slide. *C*, Same film preparation as *B*, at another place. *a*, Apparently air pores. All × 1,000.

Careful observation of the process of the film's formation suggests the following explanation. The pattern may be a result of double film layers, originating from a breaking emulsion under certain mechanical conditions. When the emulsion breaks up into droplets, a single droplet is subjected to the divergent forces of the air-liquid and the liquid-solid surfaces and to tensions within the liquid itself. At the contact between the droplet and its substratum,

interfacial tension fixes it to the solid surface, while above its center the particles still are floating freely until evaporation lowers the level

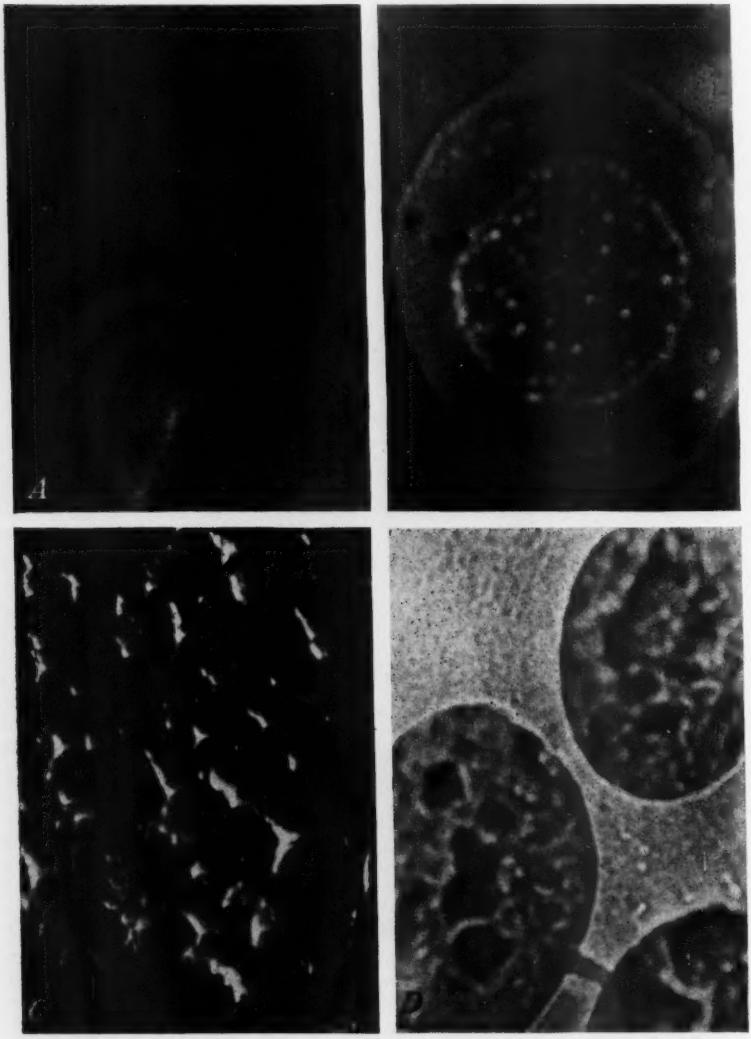

FIGURE 9.—*A*, Film of Summer-Mulsion on gelatin-coated, vertically exposed slide. × 1,000. *B*, Film of Summer-Mulsion on a methacrylate-coated slide, showing medallionlike structure. × 450. *C*, Same film preparation as *B*, showing central area of medallion; light source from above (Ultrapak). × 1,000. *D*, Film of Summer-Mulsion on agar-coated slide. × 1,000.

and these particles are also brought into close proximity with the already more solidified lower lamella of the film. Then, as the

droplet dries still further, the particles and aggregates are pulled together into a smaller area above, while the underlying material remains circularly expanded and fixed. Upon drying, the medallion-like structure results, consisting of two concentric circular plates. The exterior one appears like a frame, outlined by a cohering chain of aggregates, with a few single scattered aggregates in the outer arch. The inner circle is likewise bordered at its periphery by a beadlike bright string of aggregates. In the center a number of structural units are visible, showing some degree of attachment and grouping. The aggregates in the inner circle of the pattern are, for the most part, loosely assembled. In certain other "medallions" in the same film, the particles of the inner circles were seen to be more closely associated and linked together, even to the point of the formation of a mostly definite structure. This structure was of the nature of hexagonally-shaped homogeneous areas, surrounded and defined by borders composed of the linked aggregates.

Figure 9, *C*, shows the central area of the medallion pictured in *B*, when observed under illumination from above. Deep-seated structure is thereby revealed, and the three-dimensional character of this structure is confirmed. The image is quite appropriate for the explanation offered by Bender (*3*) for the structural formation and character of resinous films. He detected "matchlike macroparticles" present as building stones, oriented into parallel position when the film is subjected to forces of alinement.

Figure 9, *D*, gives the pattern formed by Summer-Mulsion when spread upon a surface of agar. The medallions here are oval in shape. This may be due to the upper lamellae of the film not expanding simultaneously on its entire periphery. The medallions are embedded in an anisotropic substratum. The structure of this "deeper" portion of the film is that of a dense, tight network, which in some instances is channeled between medallions in close proximity to each other. The interiors of the medallions are, on the whole, representative of the typical honeycomb arrangement observed with the materials of group 1 (oil-in-water emulsions).

MATERIALS OF GROUP 3 (MISCELLANEOUS SUBSTANCES)

Bentonite

Bentonite is a finely powdered aluminum silicate, the particles of which range in size from 10^{-4} to 10^{-2} microns. When suspended in water, it holds the liquid both mechanically (within its capillary pore spaces) and by adsorption, in the form of films over the surface of its particles. The particles of bentonite properly combined with other materials in an oil emulsion serve to increase the amount of the internally exposed surfaces of these materials. Consequently their interfacial tensions are lowered, the molecules of the different phases are anchored, and the presence of the bentonite has a stabilizing effect upon the emulsion. Since bentonite is an ingredient of the spray emulsions of group 1, it seemed desirable to examine its structure when applied alone to glass slides. This promised to give information with respect to its influence upon film formation and structure for the emulsions of which it is an incorporated substance.

Figure 10, *A*, shows the film of a 5-percent bentonite suspension in water, with illumination from below, and at high magnification.

The aggregates—for the most part small in size, light in appearance, mostly circular in shape—lie at different levels. They are, to a slight

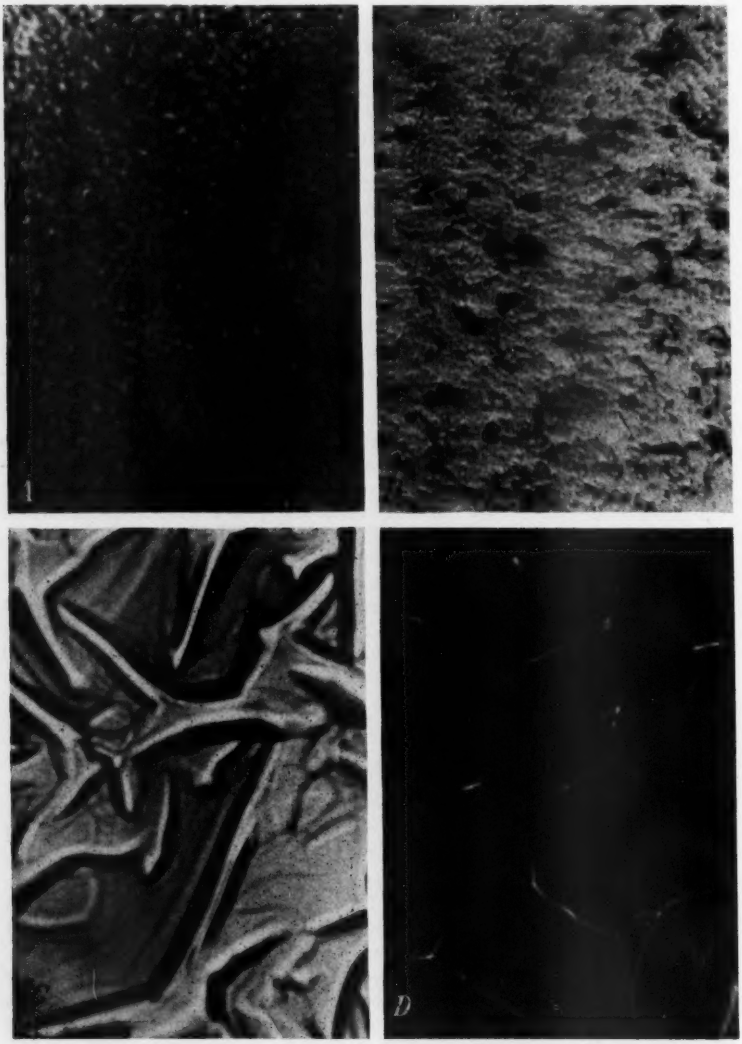

FIGURE 10.—*A*, Film of a 5-percent bentonite suspension on glass. ×1,000. *B*, Film of 3-percent bentonite suspension on glass. ×460. *C*, Film of paraffin on glass. ×1,000. *D*, Same as *C*, but at high focus. ×1,000.

extent, linked together by adhesion, and even by connecting bars, but, on the whole, they are irregularly distributed and give the general appearance of random distribution.

Figure 10, *B*, shows the film of a 3-percent bentonite suspension, under lower magnification, illuminated from above. The aspect is gross in character and the film appears to be rippled and more or less stratified. Scarcely any definitely formed (circular or otherwise) units are observable. Nevertheless, the structure is coherent, except for being interrupted here and there by fissures of various shapes and sizes, which might result from the evaporation of water.

The microscopic examinations of aqueous suspensions of pure bentonite showed their aggregates, as formed in the dry films, to be essentially like those found in the emulsions which were examined, especially those of group 1-in which bentonite was always an ingredient. However, in no pure bentonite suspension examined did the aggregates show a tendency to form a definite pattern, such as a honeycomb, lacelike, or medallionlike configuration.

Paraffin

Paraffin, unlike gelatin and agar, when spread in melted form as a film on a glass slide and allowed to cool and set gives a distinct pattern. Contraction from cooling is rapid and decisive. This results in a kind of buckling of the material, followed immediately by a heterogeneous twisting due to retroactive tensile forces. This in turn leads to the production of a film showing plastic configurations, which are sharp in contrast (fig. 10, *C*).

Figure 10, *D*, shows an area of the same film as that in figure 10, *C*, observed at the same magnification but at high focus. The three-dimensional character of the structure in *C*, has disappeared, but the contours of the highest level of the film's relief remain projected. Clearly, the surface of the film is uneven and rough. This condition would be expected to reflect itself in the structural formations of other materials, such as spray emulsions, should they be applied to it and dry thereon. This was experimentally tried and confirmed with the materials of both group 1 and group 2.

A paraffin layer, or film, is resistant to wetting. The molecular orientation of the substance is such that its hydrocarbon chains form a surface layer which practically covers the molecular groups that possess the greater attraction for water. The contact angles of the substance for water and certain organic liquids are as large as 105° (*1*).

Figure 11, *A*, shows a paraffin film, on glass, coated over with the film of an emulsion (group 1) containing 7.5 percent oil and 1 percent bentonite. The effect of the roughness of the paraffin surface is clearly apparent. The film is not continuously coherent, being absent from the elevated and sloping parts of the surface. Gravity, as was observed, exceeded the forces of adhesion and wettability as the state of surface equilibrium was being reached; the emulsion flowed and settled into the depressions and solidified, leaving the elevated part of the contour uncovered. Upon drying, a film formed in these sunken areas which adhered to the surface of the paraffin and the material took on a honeycomb aspect, not essentially different from that which characterizes a film of this emulsion on a gelatin or agar surface.

Figure 11, *B*, shows a film of Summer-Mulsion (group 2) on a paraffin surface. The aspect is quite different from that of figure 11, *A*, (material of group 1). No formal pattern, either locally or generally, is apparent. Because of the poor wettability of the substratum the liquid spray has no chance to form a coherent and complete film.

Puddles of the emulsion are scattered over the surface; they fill the depressions in the paraffin and their agglomerated particles settle down and solidify. Manifold configurations arise within these isolated areas, in consequence of the probably different physical forces present at the individual places to which the emulsion droplets are confined. The structure of these film fragments consists mostly of adhering globular oil bodies and varies in density. Coverage of the paraffin surface by the emulsion is far less complete than that observed for the emulsion from the material of group 1.

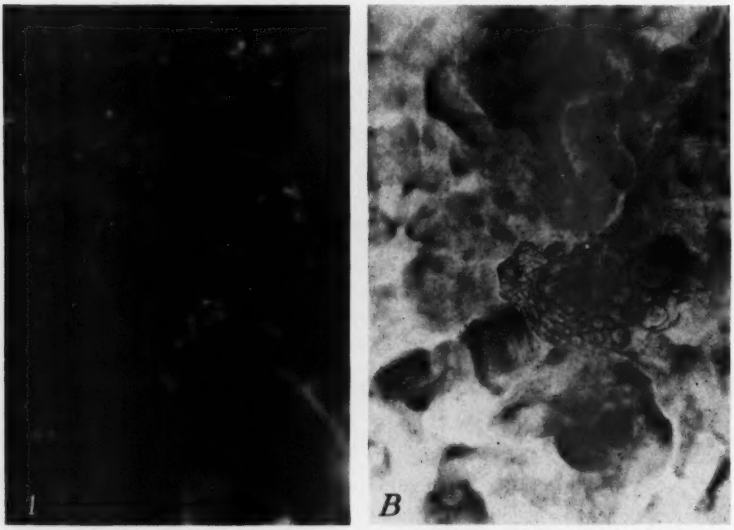

FIGURE 11.—*A*, Film of emulsion of 7.5 percent oil, 1 percent bentonite (group 1) on paraffin surface. × 1,000. *B*, Film of Summer-Mulsion (group 2) on paraffin surface. × 450.

CONCLUSIONS

This study gives evidence that films of oil emulsions possess a microscopically visible structure. Such a structure may give some information on the physical properties of the emulsion systems with respect to the degree of adherence and coverage, and to their stability; it also may reveal the distribution and the proportions of their phases.

Examination of microscopical film structures may be useful in testing oil emulsions which are intended to be applied as sprays on plant foliage. With respect to their use for reducing transpiration losses the efficiency of spray materials will depend greatly upon the permeability and adhesion of the dry film, as well as upon its extent of coverage and thickness, characteristics which can be determined partly by the patterns of their slide coatings. However, the diversity of leaf surfaces implies that a spray material may be highly effective with one species and less so with another; therefore a great variability in results is to be expected.

Observations of the behavior of spray materials on natural leaf surfaces certainly would be more informative than this study on glass

slides. However, no direct method for this purpose has been developed yet. Microscopic details of film structures on fresh leaves cannot be made visible, because the substratum on which films are applied have to be transparent in order to reveal pictures. On the other hand, treatments of leaves which might effect the desired transparency would alter the surface conditions to such an extent that any conclusions drawn from such created artifacts would be erroneous and therefore less valid than the information obtained by the method used in this study.

As to insecticidal spray materials, the contact angle and the spreading and wetting properties under existing conditions are decisive; nevertheless, the character of the configurative features of the dry film may contribute some information as to its prospective efficiency in insect control.

SUMMARY

Spray materials, which are oil-in-water emulsions, have been microscopically studied with respect to their film formation and structure on glass and other surfaces. The materials used were of two types, and were classified as group 1 and group 2.

The materials of group 1 are composed of dispersions of vegetable oils in water, with the presence of the ammonium salts of fatty acids, proteins, and bentonite, to form the system of emulsification and to give added stability. They have a high viscosity and good wetting, spreading, and quick-drying properties.

The materials of group 2 are the so-called proprietary oil emulsions. They consist of more or less refined petroleum oils, dispersed in water, and are offered in trade as insecticides. The emulsifying ingredients are not precisely specified, being trade secrets. The emulsions in this group vary in viscosity and stability. They differ from the materials of group 1 principally in the less effective character of their emulsifying and stabilizing systems.

Thinly spread layers of the emulsions of these two groups, upon receptive surfaces, dry and form adherent, solid, and more or less continuous films. Successive stages in this drying process were followed by observations with respect to the emulsions of group 1. These showed the progressive development of a fixed "honeycomb" structure, effected by aggregates of the original particles. The final distribution of phases in this honecomb structure varied with the proportional composition of the emulsion, on a given type of surface.

Successive observations on the materials of group 2 during film formation were not made. Comparison with the materials of group 1 is possible, therefore, only on the basis of the finally dried and fixed films. In general, the films of the materials of group 2 showed far less tendency, on glass surfaces, towards definite configurations, formed by aggregated particles and woven into distinctive patterns. Only under the conditions of particular ways of exposure of the surface to the spray, and the use of specially devised surfaces for the spray's reception, did formal patterns appear. Some of these were lacelike, others medallionlike, in form.

Different types of surfaces, besides glass, were prepared and used for the materials of both groups. The substratum was found to exert considerable influence on the formation of the films imposed upon it as well as on the patterns of the films.

A special case was that of a substratum of paraffin, since a paraffin film, upon solidification, develops a surface ptatern and contour of its own. Hence, besides the result as decided by the mutual reactions between surface energies of the emulsion and the substratum, there was the effect from the force of gravity which tended to localize the liquid spray in the depressions of the paraffin surface, leaving the elevated ridges uncoated. This particular effect was more pronounced with the emulsions of group 2 than with those of group 1.

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DIFFERENTIATION OF CERTAIN CRUCIFER VIRUSES BY THE USE OF TEMPERATURE AND HOST IMMUNITY REACTIONS [1]

By GLENN S. POUND, formerly *research assistant*, and J. C. WALKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

During the several years that cabbage (*Brassica oleracea* var. *capitata* L.) mosaic has been under observation in southeastern Wisconsin, it has been noted that type and severity of symptoms vary considerably with the prevailing air temperature. Initial symptoms of the mosaic disease in the field in Wisconsin usually consist of pronounced vein clearing, vein banding, and a coarse distorting mottle. As the temperature at which the plants grow gradually increases, the vein clearing and vein banding become less intense, often being completely masked, and the mottle becomes more prevalent and pronounced. Toward the end of the season, however, as the plants mature in decreasing average temperatures, the mottle symptom gradually recedes and the vein clearing and vein banding again attain prominence. Walker, LeBeau, and Pound (*16*)[2] have recently shown that these two types of symptoms are due to two distinct viruses operating together within the host plant, although evidently affected differently by high and low temperatures. The mottle symptom is due to a strain of *turnip virus* 1 Hoggan and Johnson (*3*) known as cabbage virus A, and the vein clearing symptom is due to a strain of *cauliflower virus* 1 Tompkins referred to herein as cabbage virus B (*16*). In nature these two viruses commonly occur together in host plants, at least in Wisconsin and in the Pacific Northwest, and there is no doubt that Larson and Walker (*5*) in earlier experiments were dealing with this virus combination.

In 1937 Tompkins (*10*) described a mosaic disease of cauliflower which he stated occurred in the cool coastal valleys of California. In 1938 Tompkins, Gardner, and Thomas (*15*) described a virus disease of cabbage which they named black ring. They made no study of the temperature relations of this disease but stated that it occurred chiefly in the cool coastal valleys of California during the winter months and that it was uncommon in the summer.

Walker et al. (*16*) have shown that a very close relationship exists between cabbage virus A and the black ring virus on the one hand, and between cabbage virus B and the cauliflower mosaic virus on the other hand. This paper is a report of studies directed toward the distinction of the two virus groups and of strains within each group by differential temperature reactions and by host immunity tests.

[1] Received for publication March 27, 1944.

[2] Numbers in parentheses refer to literature cited, p. 278.

MATERIALS AND METHODS

Cabbage viruses A and B used in this study were those employed in previous investigations in this laboratory (*16*). Cultures of the black ring and cauliflower mosaic viruses were supplied by Dr. C. M. Tompkins of the University of California. All four viruses were maintained in stock plants in aphidproof cages. Frequent inoculations were made to young cabbage plants in order to provide a constant supply of inoculum. All inoculations were made by rubbing leaves with absorbent cotton which had been dipped in juice extracted from diseased plants. Powdered carborundum was used regularly as an abrasive on all hosts. Greenhouses were fumigated weekly with vapors of nicotine and naphthalene to control insects.

When the reaction of the viruses on cabbage at various temperatures was being studied, young plants of Jersey Queen cabbage were inoculated with each virus or virus combination and kept in greenhouses with constant air temperatures of 16°, 20°, 24°, and 28° C. An equal number (usually 15) of healthy and inoculated plants was used at each temperature. Each experiment was repeated several times.

Reactions of all other hosts were determined by at least three separate tests with a minimum of five plants in each inoculation with each host, except in the reactions of the wild mustards to virus A and to the black ring virus, in which case only one trial each was made.

EXPERIMENTAL RESULTS

TEMPERATURE RELATIONS

Reaction of Cabbage to the Individual Viruses

It can be seen from table 1 that the incubation period of each of the four viruses increased in length as temperature decreased. At 16° and 20° C. the black ring virus characteristically produced symptoms 2 to 3 days earlier than virus A, but at higher temperatures both viruses produced symptoms concurrently. The incubation periods of virus B and cauliflower mosaic virus were practically identical. However, as will be pointed out later, virus B developed symptoms on certain cruciferous hosts 1 to 10 days later than the cauliflower mosaic virus.

Table 1.—*Effect of air temperature upon the incubation period of cabbage virus A, cabbage black ring virus, cabbage virus B, and cauliflower mosaic virus in cabbage plants in the greenhouse*

Virus	Incubation period at—			
	16° C.	20° C.	24° C.	28° C.
	Days	*Days*	*Days*	*Days*
Cabbage A	16–20	12–14	7–8	5–6
Black ring	14–18	10–12	7–8	5–6
Cabbage B	19–21	15–16	10–12	9–10
Cauliflower mosaic	18–21	14–17	10–12	9–10

A study of the symptoms produced at different temperatures revealed that the four viruses fell into two groups in regard to their reactions on cabbage at high and at low temperatures. In one group,

containing cabbage virus A and the black ring virus, mottling, stunting, distortion, and bloom reduction were very severe at 28° C. and very mild at 16°. Necrosis with the black ring virus was much more severe and pronounced at 16° and 20° than at 24° and 28°. Within this group sharp differences were noted between the reactions of virus A and the black ring virus. At 28° and 24° virus A was markedly more severe than the black ring virus, producing a severe mottle accompanied by extreme leaf distortion and severe chlorosis; the black ring virus caused much less distortion and stunting and practically no diffuse chlorosis. At 16° and 20°, on the other hand, the black ring virus was more severe than virus A, mottling and stunting were more pronounced, and necrosis was very much more common The difference in reactions of virus A and the black ring virus at high and low temperatures is of special interest because the two viruses occur in different regions. Virus A is prevalent in the midwestern and northern States in which its economic hosts are primarily summer crops. Its season of activity is therefore largely in the summer. The black ring virus occurs in the cool coastal valleys of California where economic hosts are cultivated throughout most of the year (*10*), but the prevailing air temperatures are relatively low. Moreover, Tompkins et al. (*15*) state that epidemics of the black ring disease occur chiefly in the winter and are uncommon in the summer. Although the two viruses are very similar, it may be that they have become established in their respective regions because the prevailing air temperatures in these regions are favorable for their activity.

In the other group, containing cabbage virus B and the cauliflower mosaic virus, intensity of symptom development increased with decrease in temperature. At all temperatures symptoms appeared as a chlorotic vein clearing which was prominently expressed but which rapidly became completely masked at 24° and 28° C. At 16° and 20° vein clearing and chlorotic vein banding were prominent and persistent. At these temperatures enations occurred as warty, translucent excrescences along the veins on the undersurface of the leaf more commonly than at higher temperatures. The enations, although sporadic in their development, appeared to be associated with a pronounced expression of vein clearing and vein banding. Hence they were more common at low temperatures and occurred with equal frequency with virus B and the cauliflower mosaic virus. Stunting at low temperatures, in spite of pronounced symptoms, was no greater than at higher temperatures and was less than that caused by virus A and the black ring virus. The effect of temperature upon disease development for the cauliflower mosaic virus was exactly parallel to that for virus B. The two viruses differed in that symptoms of the cauliflower mosaic virus were always milder than those of virus B. The fact that B-infected plants were more chlorotic than those infected with the cauliflower mosaic virus often served as a sharp point of distinction between the two viruses.

Reaction of Cabbage to Various Combinations of the Viruses

When viruses A and B were inoculated simultaneously into the same plants, the degree of interaction was somewhat clearly defined by the temperature effect on symptom expression of the virus combination. An examination of figure 1 will show that the combined

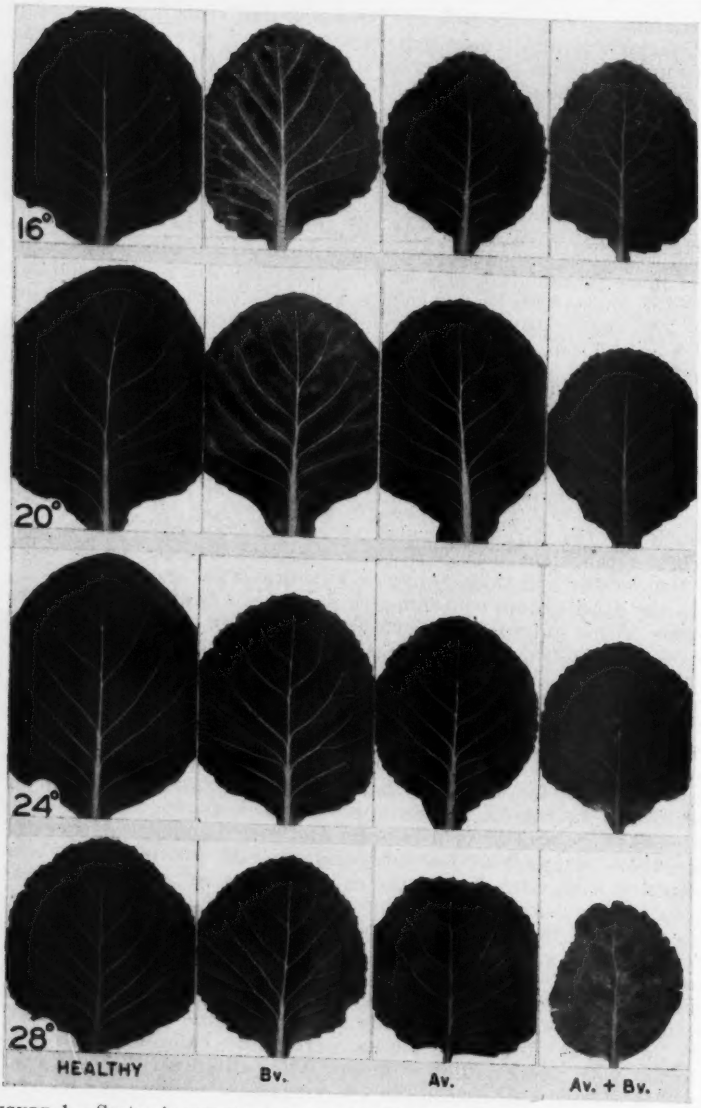

FIGURE 1.—Systemic symptoms on young leaves of Jersey Queen cabbage produced by cabbage virus B (Bv.), cabbage virus A (Av.), and cabbage viruses A and B (Av.+Bv.) together at various temperatures. Note that symptoms of virus B are masked at 28° and 24° C. but are prominently expressed at 20° and 16°C. Note also the progressive increase in severity of symptoms of virus A with increase in temperature and the pronounced increased severity at 28° and 24° when viruses A and B occur together.

effect of the two viruses was more severe than that of either virus alone. At higher temperatures the addition of the B component to virus A resulted in the appearance of vein clearing and rapidly changed the coarse chlorotic mottle into a fine mottle with much more chlorosis and increased severity, with the result that ultimate symptoms bore little resemblance to those of either virus alone. Stunting, leaf distortion, bloom reduction, and premature leaf abscission were very severe. Necrosis, quite uncommon to virus A, became very severe on some plants. Symptoms (other than stunting and chlorosis) of virus B alone at 24° and 28° rapidly became masked, but the presence of this virus in combination with virus A at these temperatures effected a striking change in the type and severity of symptoms produced. Whether the presence of this virus enhanced the activity of virus A, or vice versa, or whether this symptom change was the additive result of the effect of each individual virus on the metabolism of the host is a question prompting further investigation. At 16° and 20° the combined effect of viruses A and B resulted in more stunting and chlorosis than for either virus alone but there was no change in type of symptoms as at 28°. The effect of each virus was evident, and there was little apparent interaction between them. The activity of virus A was reduced such that little additive effect was evident, and the ultimate symptoms were largely those of virus B.

A few days after plants showing severe A+B symptoms were moved from a house at 28° to one at 16° C., subsequently developing leaves showed only a very mild mottle and a vein clearing which gradually increased in prominence. Symptoms on young leaves showed little resemblance to those on plants kept at 28°. Likewise, a few days after A+B plants showing typical low-temperature symptoms were transferred from 16° to 28°, the conspicuous vein clearing and vein banding disappeared, and young leaves showed a severe fine mottle accompanied by much stunting and distortion. When these two groups of plants were again removed to the houses of their original incubation, they once again developed symptoms characteristic of that specific temperature. These reversible temperature reactions of plants infected with both viruses fully confirm observations made of the disease in the field.

When plants showing systemic symptoms of virus A were reinoculated with virus B, or vice versa, there developed at each temperature symptoms typical of the A+B combination after the respective incubation period of the second virus introduced. Furthermore, the two viruses were easily recovered individually from such plants by the use of heat inactivation and differential hosts as described by Walker et al. (*16*). The fact that there was no immunization of the host by virus A toward virus B and vice versa would indicate no close relationship between the two viruses.

Symptoms produced by the mixture of cabbage A and cauliflower mosaic viruses (fig. 2) were practically identical with those described for the A+B combination. Just as symptoms of the cauliflower mosaic virus alone on cabbage were slightly milder than those of virus B, the symptoms produced by this virus in combination with virus A were slightly milder than those of the corresponding A+B combination.

Combinations of the black ring virus with virus B and with the cauliflower mosaic virus were very similar to the corresponding com-

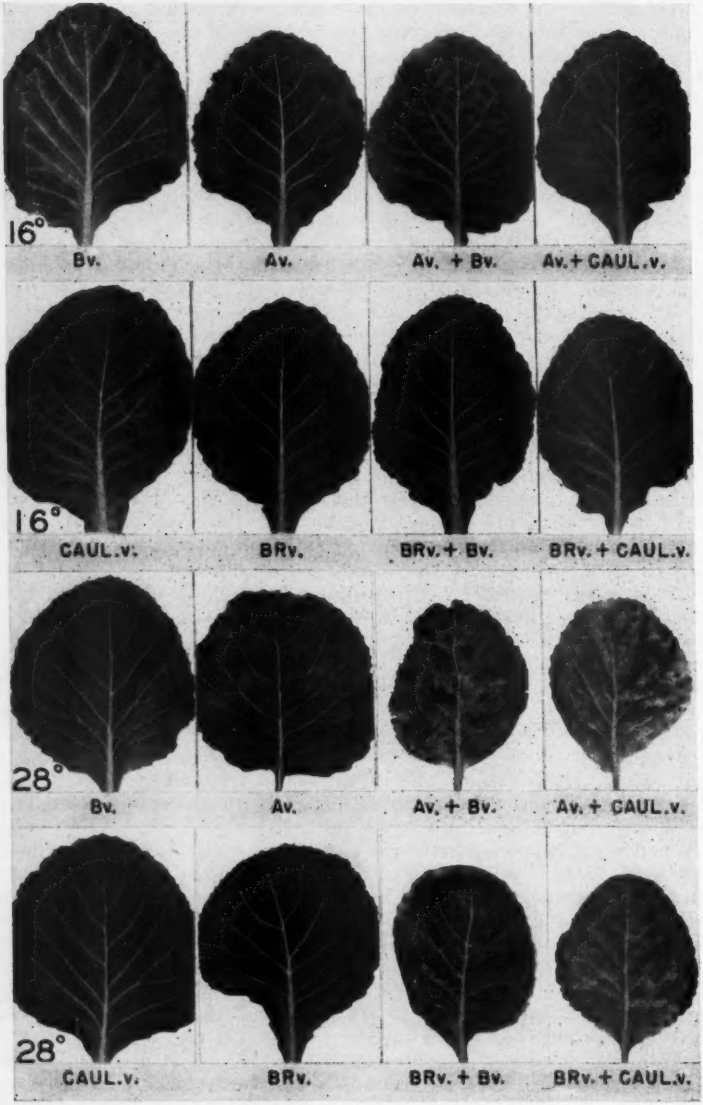

FIGURE 2.—Symptoms produced on young leaves of Jersey Queen cabbage by cabbage A (Av.), cabbage B (Bv.), cabbage black ring (BRv.), and cauliflower mosaic (Caul. v.) viruses alone and in various combinations at 16° and 28° C. Note the similarity in symptoms of virus A and the black ring virus both when alone and in combination with virus B or the cauliflower mosaic virus. Note also the similarity of symptoms of virus B and the cauliflower mosaic virus.

binations of virus A except that the differences in the temperature reactions of virus A and the black ring virus were also manifested in the combinations of these viruses. At 24° and 28° the black ring virus combinations produced less chlorosis and less stunting than did the corresponding combinations of virus A. In addition, dark-green rings and ring spots typical of the black ring virus were manifested. At 16° and 20° all combinations of the black ring virus produced more necrosis and a slightly more prominent mottle than those of virus A.

Thus in the various combinations involving viruses of the two groups the reactions of the cauliflower mosaic virus were practically identical with those of virus B, and the reactions of the black ring virus were very similar to those of virus A (fig. 2). These similarities were expected since the same relations were manifested in the reactions of each of the viruses alone. Tompkins (*10*) and Tompkins et al. (*15*) made no mention of the black ring and cauliflower mosaic viruses occurring together within the same plant in nature. Neither did they describe symptoms typical of the combined action of the two viruses.

Reaction of Selected Hosts at Various Temperatures to Cabbage Virus A and the Black Ring Virus

When cabbage virus A and the black ring virus were studied over an extensive host range (*16*) certain hosts were noted on which the two viruses acted somewhat differently. These were studied further at 16°, 20°, 24°, and 28° C. to determine which could be used for cross-immunity tests at one or another temperature.

Nicotiana glutinosa L.

The reactions of the two viruses on *N. glutinosa* differed only in minor respects, but the effect of temperature upon symptom expression was most striking. With both viruses the effect upon the host plant was most severe at 16° and 20° and least so at 28°, a temperature reaction quite the reverse of that noted for cabbage. Moreover, the type of symptom changed with increase in temperature. At 16° primary symptoms were necrotic flecks with chlorotic halos, while systemic symptoms were vein clearing, mottle, stunting, and necrosis. At 20° the symptoms were of the same general type but more severe. At 16° and 20° black ring symptoms were slightly more severe than those of virus A. At 24° there was a shift to more mottle, less necrosis and less stunting. At 28° primary symptoms were small necrotic flecks around which conspicuous zonate rings slowly developed. Similar ring spot symptoms occurred systemically (fig. 3.) The youngest leaves showed no symptoms (even after 90 days' incubation), and it was only when leaves were separated from the growing point by four to six internodes that symptoms appeared. Repeated attempts to recover the viruses from the symptomless young leaves were unsuccessful, even after 50 days' incubation. Recovery tests from leaves showing symptoms yielded the viruses in fair concentration. No necrosis, mottling, or distortion occurred at this temperature.

Thus on this host completely different types of symptoms were produced at different temperatures, the mildest symptoms occurring at 28° C. Since *Nicotiana glutinosa* was favored by relatively high

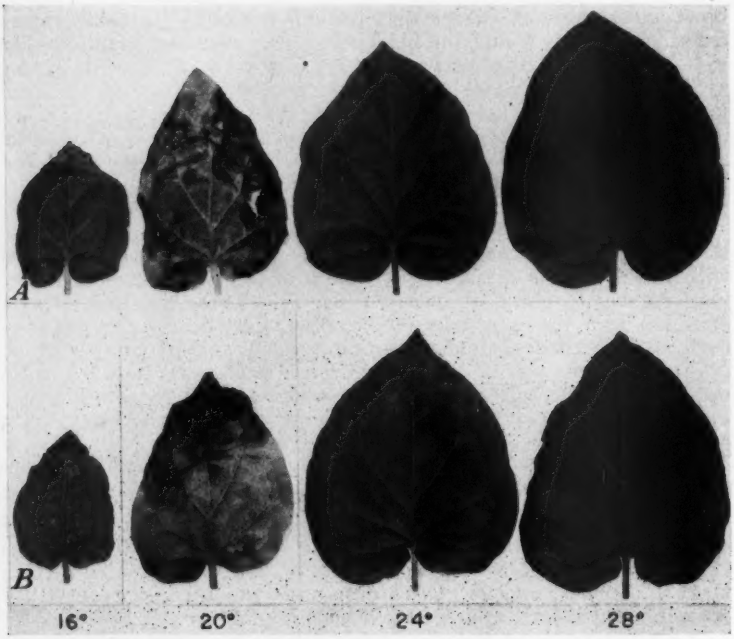

FIGURE 3.—Systemic symptoms produced by (*A*), virus A, and (*B*), black ring virus, on *Nicotiana glutinosa* at temperatures of 16°, 20°, 24°, and 28° C., respectively. All leaves are of the same age. Note the extreme similarity in the reactions of the two viruses and the effect of temperature upon symptom "type" as well as decrease of severity with increase in temperature. Compare reaction of this host to virus A with the reaction of cabbage to the same virus (fig. 1), where the disease increases in severity with increase in temperature.

temperatures, and since each virus produced its greatest effects at temperatures unfavorable to the host and its least effects at temperatures favorable to the host, it would seem that the temperature effect upon the host-virus relationship was largely that of the temperature effect upon the host. This condition has its counterpart in the effect of temperature on the reaction of cabbage to the A and black ring viruses. Cabbage makes much better growth at 16° and 20° than at 28° C. It will be remembered that the symptom severity of the two viruses on cabbage decreased with decrease in temperature except for the increase in necrosis with the black ring virus at 20°. It is often stated that plants in a vigorous growing condition develop more severe symptoms than plants in a less active state. If this be true, then the reaction of *N. glutinosa* is exceptional, both in the mild symptoms produced and in the slowness with which the two viruses invaded actively growing young leaves at 28°.

It is evident that there must be also a temperature effect upon the activity of the viruses themselves. It was pointed out earlier that both viruses show incubation periods on cabbage, a low temperature plant, which increase in length with decrease in temperature. On tobacco, a high temperature plant, the same viruses also show incu-

bation periods which increase in length with decrease in temperature. However, as will later be shown, symptoms on *Nicotiana rustica* appear concurrently at low and high temperatures. Furthermore, on many hosts virus A and the black ring virus do not show the same temperature reaction in regard to the length of the incubation period. Walker et al. (*16*) pointed out that the black ring virus produces more necrosis than does virus A. This production of necrosis, especially on cruciferous plants, is restricted almost entirely to temperatures below 24° C. On many hosts this virus is more severe at 20° than at 28°, whereas virus A almost without exception is most severe at 28°. These facts clearly indicate that, as regards temperature effects, the reaction on any host is due to the temperature effect upon the specific host-virus combination and maybe due to the temperature effect upon the host, the virus, or both. The last of these three possibilities is probably the one which usually prevails.

Nicotiana rustica L.

When *Nicotiana rustica* was used the symptoms at all four temperatures appeared almost simultaneously. At 24° and 28° differences between the two viruses were slight. The black ring virus produced primary circular chlorotic lesions at 24° and 28°; virus A produced them indistinctly and only at 24°. Both produced zonate chlorotic rings and solid chlorotic spots systemically. At 16° and 20° the reactions of the two viruses were markedly different. The black ring virus produced numerous necrotic primary lesions and numerous chlorotic systemic lesions, 4 to 5 mm. in diameter and bordered by necrotic dots; stunting and leaf distortion were marked. The primary lesions caused by virus A were largely scattered chlorotic lesions while the systemic lesions were larger, fewer in number, and stunting was less pronounced. The systemic lesions of the two viruses are compared in figure 4.

Nicotiana multivalvis Pursh

On *Nicotiana multivalvis* the differences were most pronounced at 16° C., where virus A produced no primary symptoms while the black ring virus produced a conspicuous pattern of small white rings and ring spots. Systemically the symptoms were most severe at 16°, decreasing with increase in temperature as with *N. glutinosa* but without any marked change in symptom type. The black ring virus was the more severe at 16°, the difference becoming less pronounced with increase in temperature.

Wild Mustards

The effects of the two viruses on young plants of *Brassica juncea* (L.) Coss. (Indian mustard), *B. nigra* (L.) Koch (black mustard), *B. arvensis* (L.) Ktze. (charlock), and *B. campestris* L. (wild yellow mustard) at 20° were easily distinguishable (fig. 5). The black ring virus produced numerous, angular necrotic lesions on inoculated leaves. Necrosis spread, became systemic, and plants died within 3 weeks after inoculation. Except on charlock, virus A produced no primary necrotic lesions, and plants were not killed until several days

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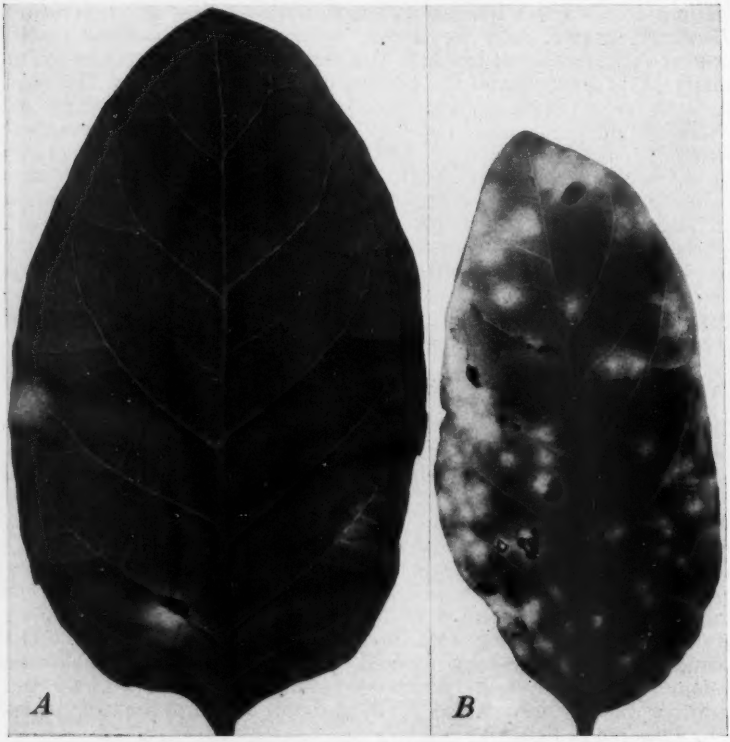

FIGURE 4.—Systemic symptoms produced on *Nicotiana rustica* at 16° C.: *A*, Cabbage virus A; *B*, Black ring virus. Note the more pronounced mottle and more severe stunting caused by the black ring virus.

after death occurred to black-ring-infected plants. Symptoms were markedly milder than those produced by the black ring virus. At 28° disease development was so rapid with both viruses that plants were killed within 5 to 8 days after inoculation.

Brassica oleracea var. *gemmifera* DC. (Brussels sprouts)

At 16° and 20° C. symptoms of the two viruses on Brussels sprouts (var. Long Island Mammoth) were markedly different. The black ring virus produced a severe necrosis on inoculated leaves as circular lesions which expanded and coalesced, causing the entire leaf to die. Systemic symptoms appeared as circular chlorotic rings and spots which rapidly became necrotic to produce a conspicuous pattern of black rings and streaking of the veins. Virus A produced only chlorotic lesions, or no symptoms at all, on inoculated leaves and a systemic mottle which was very mild and never became necrotic until 4 to 5 weeks after inoculation. At 20° the effects of the two viruses were so different that they might easily have been considered as two distinct diseases (fig. 6). At 28° the symptoms of the two viruses were indistinguishable.

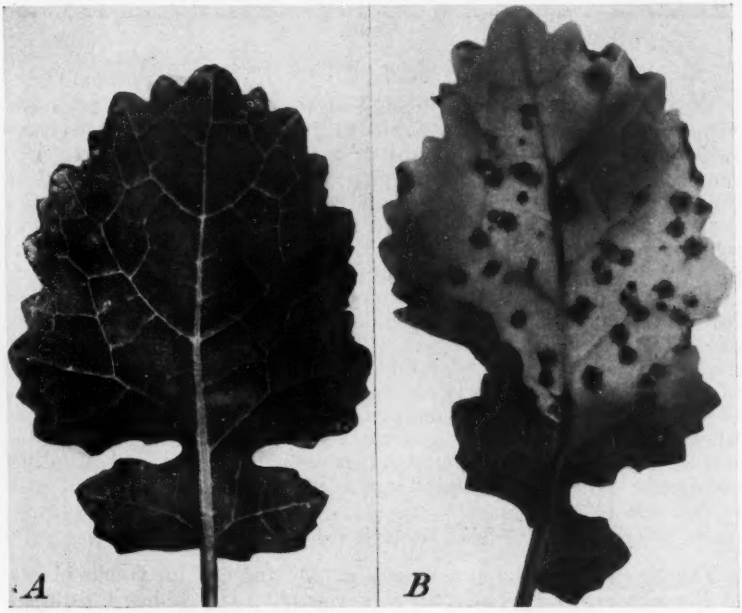

FIGURE 5.—Symptoms produced on inoculated leaves of Indian mustard (*B. juncea*) at 20° C.: *A*, Virus A; *B*, black ring virus. Note the extreme difference in the effects produced by the two viruses.

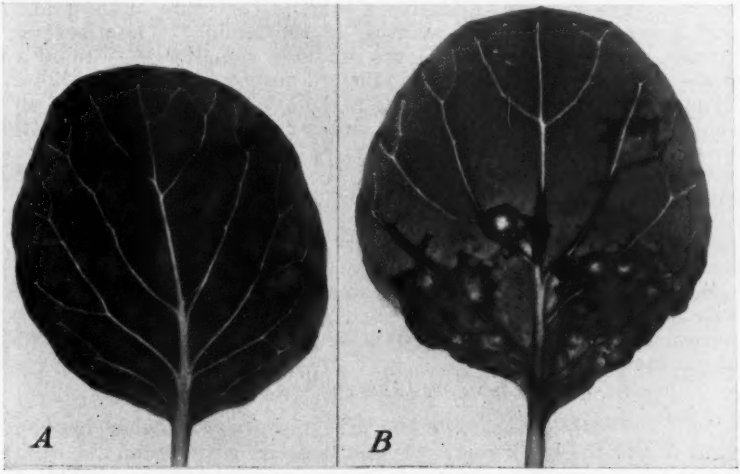

FIGURE 6.—Systemic symptoms produced on Brussels sprouts at 20° C.: *A*, Mild mottle caused by virus A; *B*, Severe necrotic pattern produced by the black ring virus.

REACTION OF VARIOUS HOSTS TO CABBAGE VIRUS B AND CAULIFLOWER MOSAIC VIRUS

Brassica pekinensis (Lour.) Rupr. (Chinese cabbage)

On Chinese cabbage (var. Chihli) at 16° C. the cauliflower mosaic virus produced symptoms after 18 to 21 days as a conspicuous chlorotic vein clearing. This symptom became progressively more pronounced, and leaf midribs developed a marked curvature. Leaf laminae became severely ruffled and stunted, resulting in a conspicuous rosette in which a few old leaves showed no malformation, while severely stunted and distorted young leaves formed the central cluster. Virus B produced symptoms some 7 to 10 days later than the cauliflower mosaic virus. These appeared as vein clearing and leaf curvature and were not unlike the symptoms caused by the cauliflower mosaic virus except that severe stunting and rosetting did not occur. These much milder symptoms served as a sharp point of distinction between the two viruses. At 20° and 24° the symptoms of the two viruses became increasingly more alike and at 28° they were indistinguishable. In general, rosetting and vein clearing decreased with increase in temperature while chlorosis and stunting increased. As a means of differentiating the two viruses this host served best at 16°.

Wild Mustards and Rape

The reactions of the two viruses at 20° and 28° on young plants of *Brassica arvensis* (charlock), *B. campestris* (wild yellow mustard), *B. juncea* (Indian mustard), *B. hirta* Moench, (*B. alba* (L.) Rabenh. (white mustard)) and *B. napus* L. (rape var. Dwarf Essex) were studied.

At 20° symptoms of the cauliflower mosaic virus became apparent in 12 to 15 days as a conspicuous chlorotic vein clearing and mild mottle. Young leaves exhibited a marked curvature of the midrib and a severe ruffling and stunting of the lamina. These severely stunted and inrolled young leaves produced a marked rosette on all hosts. Many plants of the wild mustard hosts were ultimately killed. Symptoms of virus B appeared 2 to 4 days later than those of the cauliflower mosaic virus as vein clearing followed by mild mottle with curving and wrinkling of the leaves. No marked rosette of young leaves developed on any of the hosts, and symptoms were much milder than those caused by the cauliflower mosaic virus.

At 28° the cauliflower mosaic virus again produced symptoms earlier and much more severe than did virus B. Stunting, distortion, mottling, and chlorosis were more severe, but rosetting was less pronounced than at 20°. Symptoms were very severe and most plants were finally killed. At both 28° and 20° the two viruses were easily separable by the degree of symptom severity rather than by the type of symptom.

HOST IMMUNITY REACTIONS IN CABBAGE

In the preceding section differential temperature reactions between virus A and the black ring virus and between virus B and the cauliflower mosaic virus on several hosts were described. As a result of these critical studies of the effects of temperature on symptom expression, certain host reactions were found which differentiated between the viruses in question so distinctly that they were selected

for use in cross-immunity tests. The efficacy of these selected hosts in differentiating between the viruses in question depended upon the use of a temperature which insured the expression of the differentiating symptoms. Although the differentiation was more distinct at one specific temperature, the hosts used were tested at all four temperatures to widen the scope of the test.

Specific Immunity Against the Black Ring Virus Produced by Virus A

Immunological tests involving virus A and the black ring virus were set up as follows: Each virus was inoculated to 10 cabbage plants. After all plants developed systemic symptoms the A-infected plants were reinoculated with the black ring virus, and the plants infected with the black ring virus were reinoculated with virus A, inoculations being made on systemically infected leaves. At the same time 10 healthy plants of the same age were inoculated (to serve as controls) with each of virus A, the black ring virus, and a mixture of the 2 viruses. Two weeks after systemic symptoms appeared in the 3 groups of control plants inoculations were made from all of the 5 groups of 10 plants each to *Nicotiana multivalvis*, *N. rustica*, and *Brassica oleracea* var. *gemmifera* at temperatures of 16°, 20°, 24°, and 28°. Extracts were taken from young leaves above inoculated leaves.

Symptoms produced on plants inoculated with extracts taken from the A-infected plants reinoculated with the black ring virus agreed in every respect with symptoms produced on these hosts by virus A alone. On all hosts at all temperatures symptoms showed no resemblance to those of the black ring virus but, on the contrary, were exactly identical with those of virus A as described in the preceding section. Correspondingly, extract from cabbage plants infected with the black ring virus and reinoculated with virus A produced symptoms exactly like those of the black ring virus alone. This, of course, was expected since the more severe symptoms of the black ring virus would cover up the mild symptoms of virus A if virus A were present. Figures 7 and 8 show the results of the inoculations to Brussells sprouts and *N. rustica*, respectively.

Inoculum taken from plants infected with the A-black-ring mixture produced symptoms similar to those of the black ring virus alone. This indicated that virus A does not inhibit the establishment of the black ring virus when the two are introduced into the plant simultaneously.

In another test 40 young Brussels sprouts plants were inoculated with virus A and incubated at 20°. After plants developed systemic symptoms, 20 of the infected plants were reinoculated with the black ring virus. At the same time 20 healthy plants of the same age were inoculated with the black ring virus. After 10 days the healthy plants inoculated with the black ring virus had developed a severe necrosis on inoculated leaves, and subsequent systemic necrosis was severe and typical of the black ring virus. Plants which were infected with virus A and reinoculated with the black ring virus developed no necrosis on inoculated leaves and no systemic necrosis until several days after systemic necrosis appeared in the black ring control plants.

In still another test 10 plants of *Nicotiana multivalvis*, infected with

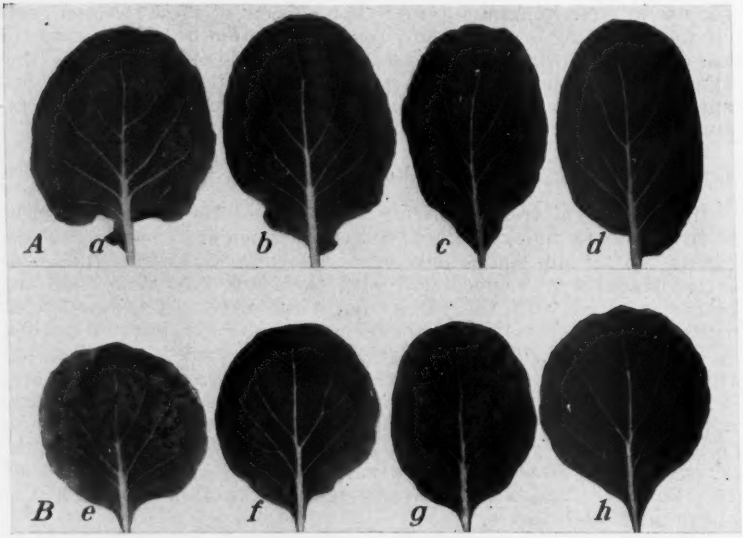

FIGURE 7.—*A*, Systemic symptoms produced on Brussels sprouts at 16° C. when inoculated with extracts taken from cabbage plants used in the cross-immunity tests of virus A and the black ring virus. *A*, Leaves from plants inoculated with: *a*, Extract taken from cabbage infected with black ring virus alone; *b*, extract from cabbage infected with virus A alone; *c*, extract from cabbage infected with black ring virus and reinoculated with virus A; *d*, extract from cabbage infected with virus A and reinoculated with the black ring virus, respectively. *B*, primary symptoms produced on inoculated leaves (*e*, *f*, *g*, *h*) of same plants.

virus A and showing only a mild systemic mottle, were inoculated with the black ring virus. At the same time an equal number of healthy plants of the same age were inoculated with the black ring virus. After 5 days at 24° the healthy plants inoculated with the black ring virus developed numerous white rings and ring spots on inoculated leaves. Plants infected with virus A and reinoculated with the black ring virus failed to develop primary symptoms.

These tests would indicate a strain relationship between virus A and the black ring virus if the failure of the black ring virus to establish itself in plants infected with virus A were due to a specific acquired immunity.

SPECIFIC IMMUNITY AGAINST VIRUS A PRODUCED BY THE BLACK RING VIRUS

In an extensive host range study of virus A and the black ring virus (*16*) one host was found which appeared to be completely differential for the two viruses. This host, *Solanum integrifolium* Poir. (Chinese scarlet eggplant), was found to develop conspicuous, necrotic, primary lesions when inoculated with virus A at 28°, 24°, and 20°. This necrosis became systemic and appeared as numerous lesions 2 to 4 mm. in diameter and as streaking of the veins on young leaves. Leaf abscission resulting from the necrosis was marked. No mottle sympton developed. In four separate trials the black ring

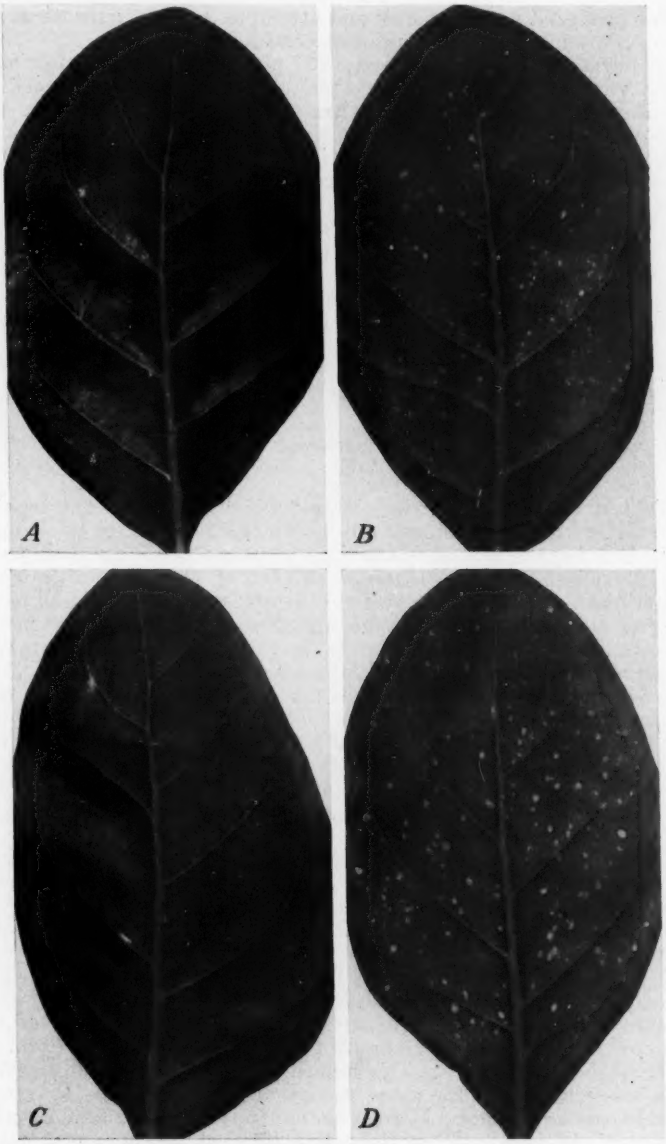

FIGURE 8.—Symptoms produced on inoculated leaves of *Nicotiana rustica* at 16° C., with extracts taken from cabbage plants used in cross-immunity tests of virus A and the black ring virus. Leaves taken from plants inoculated with: *A*, Extract taken from cabbage infected with virus A alone; *B*, extract from cabbage infected with black ring virus alone; *C*, extract from cabbage infected with virus A and reinoculated with the black ring virus; *D*, extract from cabbage infected with black ring virus and reinoculated with virus A.

virus produced no symptoms, and attempts to recover the virus from the symptomless plants were unsuccessful.

No symptoms were produced on 27 plants of *Solanum integrifolium* with virus taken from cabbage plants infected with the black ring virus and reinoculated with virus A. Neither did extracts from the plants infected with the black ring virus alone produce symptoms. Virus taken from cabbage plants infected with virus A and reinoculated with the black ring virus produced symptoms similar to those of virus A alone (fig. 9). Inoculum taken from cabbage plants infected with the A-black-ring mixture produced symptoms similar to those produced by virus A, indicating that the black ring virus does not inhibit the establishment of virus A in plants when the two viruses are introduced into the plants simultaneously. Thus in this test (fig. 9, *C*) the black ring virus did not prevent the establishment of virus A in systemically infected cabbage plants. However, when cabbage plants were first inoculated with black ring virus and then reinoculated with virus A the latter produced no symptoms (fig. 9, *D*).

Specific Immunity Against Cauliflower Mosaic Virus Produced by Virus B

To test the ability of virus B to protect cabbage plants against infection with the cauliflower mosaic virus a test similar to that just described for the A and black ring viruses was used. Ten young cabbage plants were inoculated with each of virus B and the cauliflower mosaic virus. After systemic symptoms appeared in all plants the B-infected plants were reinoculated with the cauliflower mosaic virus, and the plants infected with the cauliflower mosaic virus were reinoculated with virus B, At the same time virus B, cauliflower mosaic virus, and a mixture of the 2 viruses were each inoculated to 10 healthy plants of the same age. Two weeks after systemic symptoms appeared in the 3 groups of control plants, inoculations were made from each of the 5 groups of 10 plants to Chinese cabbage plants at 16°, 20°, 24°, and 28° C., a minimum of 5 test plants being used in each inoculation. The experiment was repeated 3 times and gave the same results each time.

Symptoms produced by an extract taken from B-infected plants which were reinoculated with cauliflower mosaic virus were identical at each temperature with those produced by virus taken from plants infected with virus B alone. Symptoms produced by extracts taken from plants infected with the cauliflower mosaic virus alone and from plants infected with the cauliflower mosaic virus but later reinoculated with virus B were identical. Inoculum taken from cabbage plants infected with a mixture of the 2 viruses produced symptoms characteristic of the cauliflower mosaic virus. In figure 10 are shown the results obtained in one of the experiments. One month later a new series of inoculations was made from the same groups of cabbage plants to young Chinese cabbage plants. Results similar to those in the first inoculations were obtained. These tests show that cabbage plants systemically infected with virus B are protected by the virus against infection by the cauliflower mosaic virus and indicate a strain relationship between virus B and the cauliflower mosaic virus.

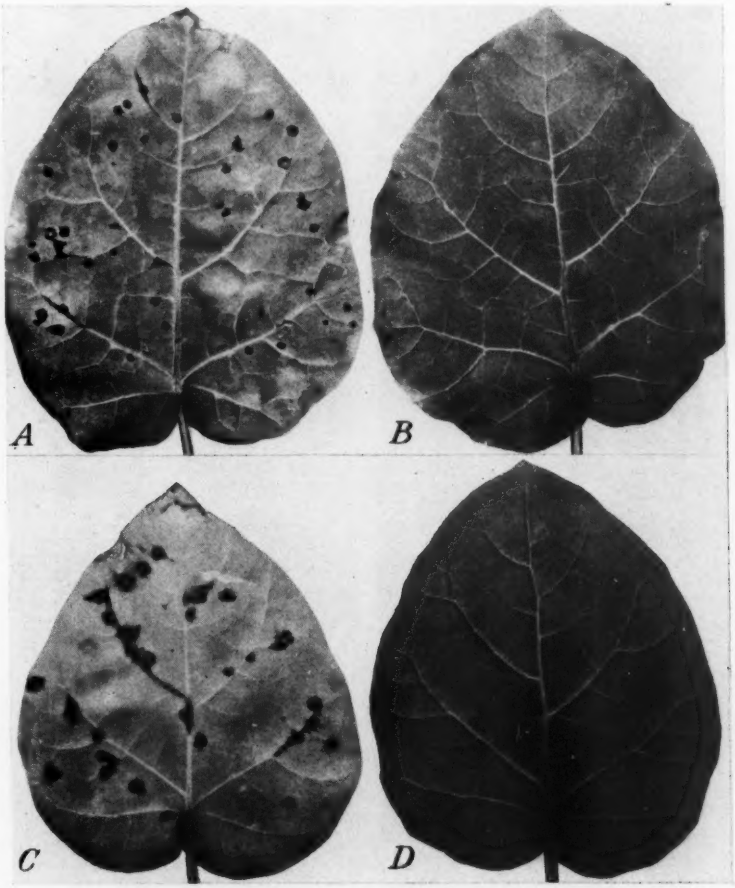

FIGURE 9.—Symptoms produced on inoculated leaves of *Solanum integrifolium* with extracts taken from cabbage plants used in the cross-immunity tests of the A and black ring viruses. Leaves from plants inoculated with: *A*, Extract taken from cabbage infected with virus A alone; *B*, extract from cabbage infected with black ring virus alone; *C*, extract from cabbage infected with virus A and reinoculated with the black ring virus; *D*, extract from cabbage infected with black ring virus and reinoculated with virus A. Note that the black ring virus produced no symptoms.

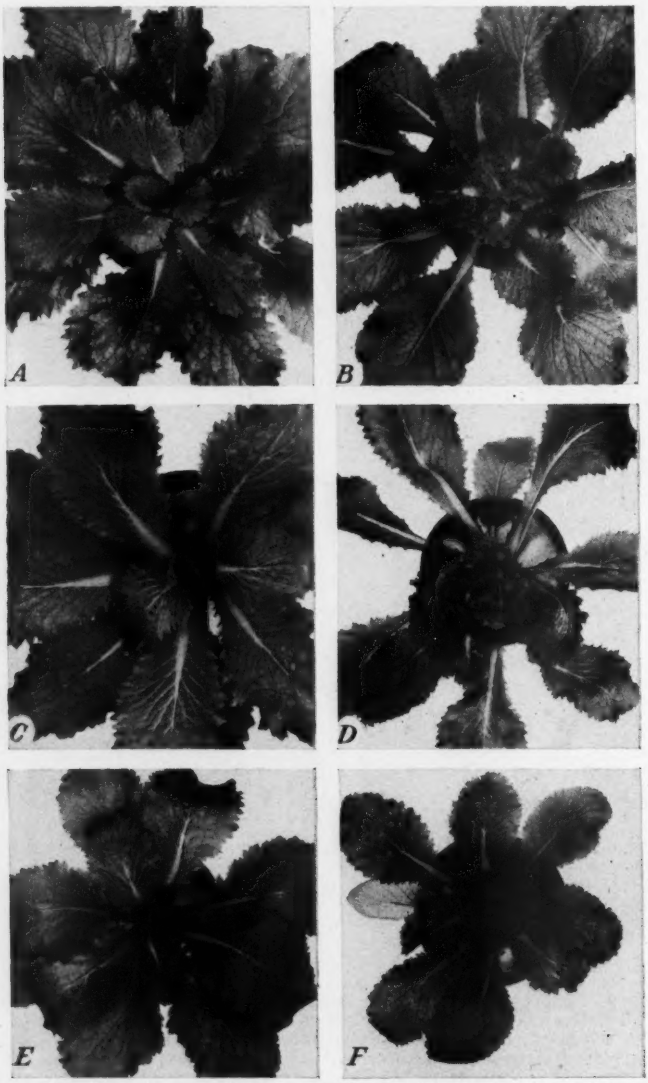

FIGURE 10.—Symptoms produced on Chinese cabbage at 20° C. 30 days after inoculation with extracts taken from cabbage plants used in cross-immunity tests of virus B and the cauliflower mosaic virus. *A*, Extract taken from cabbage infected with virus B alone; *B*, extract from cabbage infected with the cauliflower mosaic virus alone; *C*, extract from cabbage infected with virus B and reinoculated with cauliflower mosaic virus; *D*, extract from cabbage infected with cauliflower mosaic virus and reinoculated with virus B; *E*, uninoculated control; *F*, extract from cabbage inoculated with a mixture of virus B and cauliflower mosaic virus. Note that extract taken from plants infected with virus B reinoculated with cauliflower mosaic virus produces symptoms indistinguishable from those of virus B alone.

DISCUSSION

The effect of temperature upon host-virus relationships has received disproportionately little attention in comparison with other phases of virus investigations. Many comparisons of viruses today are based upon symptoms and properties of the viruses. Evidence presented in this paper shows that type and intensity of symptoms may depend greatly upon the temperature to which the host plants are exposed. Other experiments under way indicate that temperature may radically alter the activity and concentration of the virus within the host. Since the determination of the physical properties of the viruses is influenced by the concentration of the virus in the expressed sap, it is suggested that the temperature under which they develop in the host may indirectly affect the physical properties of the virus as ordinarily determined. Thus in making comparison in symptomatology and properties of viruses prime consideration should be given to the temperatures of the environments in which the symptoms develop and the viruses multiply. High temperature is often reported to reduce the severity of a given virus. Observations reported herein indicate that the effect of temperature upon disease severity depends upon the specific host-virus complex and may be due to the temperature reaction of the host, virus, or both.

The effect of temperature upon host-virus reaction may have considerable bearing on virus classification and nomenclature. The inadequacies of a system of classification based primarily upon symptomatology are revealed by the reactions of cabbage viruses A and black ring on *Nicotiana glutinosa* and other hosts at different temperatures. For instance, the symptoms of these viruses on *N. glutinosa* at 28° C. include those given by Holmes (*4*) for his family, Annulaceae, while the symptoms produced on this host at 16° are typical of those given for the family Marmoraceae. The name black ring is very appropriate for the reactions of the black ring virus on crucifers at low temperatures but very inappropriate for symptoms at high temperatures.

Many investigators have tested numerous host plants with certain viruses in the hope of finding hosts which would give a differential reaction to related viruses or a local-lesion reaction which could be used in quantitative measurement of the viruses. Results given in this report show that by testing related viruses on selected hosts at various temperatures differential reactions may be obtained which may be more marked than those obtained by testing the viruses on a large number of hosts at only one temperature. Likewise, temperature studies may provide a local-lesion reaction which could not be found by trying several hosts at only one temperature. These values of temperature studies are well illustrated by the reaction of *Nicotiana rustica* to the cabbage A and the black ring viruses. At high temperatures the two viruses were not easily distinguishable on this host, but at low temperatures their reactions were very different. Furthermore, the reactions of the black ring virus at 16° and 20° were such that they could be easily applied to quantitative studies of the virus itself. Many hosts were found in this and other investigations (*16*) that gave a local-lesion reaction to this virus at low temperatures but not at high temperatures. Most of the differentiating symptoms used

in the immunity reactions reported in this paper resulted from finding differential temperature reactions of the viruses in question.

The method of measuring specific acquired immunity employed in this investigation proved just as effective as the ordinary local-lesion-mottle test although it involved more work. Its effectiveness shows that where two related viruses have a differential reaction on any host their ability to immunize plants against each other can be tested in any plant in which they develop systemically.

Significant features in the properties and host range of various crucifer viruses are presented in table 2. These comparisons are made in order to focus attention upon possible relationships in this heterogeneous group of viruses. Although the writers are of the opinion that these viruses fall into two distinct groups, it is realized that any such relationships can be truly ascertained only by parallel studies in the same laboratory. However, the viruses do show enough common characteristics to justify a limited amount of speculation regarding their relationships.

A study of table 2 reveals that in properties the first 14 viruses listed fall more or less into 1 group, the properties of which are limited by the following values: Aging in vitro, 48–384 hours; dilution, 1–600 to 1–100,000; thermal inactivation, 50°–68° C. The greatest variation in these properties lies in the measure of dilution tolerance. The fact that different test plants were used might account for some of this variation. It is the writers' experience that, with cabbage virus A, infection on tobacco can be obtained at a lower concentration than on cabbage. This may be due to chance since much more leaf area is covered in inoculating 10 tobacco plants than in inoculating 10 cabbage plants. Furthermore, in only 1 trial was 1–100,000 the dilution inactivation point; and in each case in which inactivation was reached only at 1–50,000, the highest value at which infection occurred was 1–10,000. The property which varies the least is thermal inactivation. Ten of the fourteen viruses have inactivation points within 6° of each other. With 3 of the remaining 4 viruses inactivation was obtained by heating at intervals of 3° to 5°. Had closer measurements been made this variation would probably have been reduced.

In regard to host range, the 14 viruses show many points of similarity, and yet each differs from the others. All infect one or more solanaceous and chenopodiaceous hosts. Local necrotic lesions are produced on tobacco by all but the T_1 strain of turnip mosaic (*7*). The virus of radish mosaic (*13*) is unique in that it becomes systemic in this host. Within the Cruciferae it is significant that only virus A, black ring virus, ring necrosis virus (*6*), the T_8 strain of turnip mosaic (*7*), the turnip virus of Chamberlain (*2*), and the radish mosaic virus (*13*) are widely pathogenic on subspecies of *Brassica oleracea*, although cabbage was the only subspecies tested with turnip virus 1 (*3*). Of the turnip mosaic viruses only the strain of Chamberlain (*2*) was pathogenic on wallflower; all were pathogenic on black mustard and rape except Tompkins' strain; only strain T_8 and T_9 (*7*) affected kohlrabi. Neither of the stock viruses (*12*) was pathogenic on members of *B. oleracea*. The mild stock mosaic virus affected wallflower.

In view of the points of similarity between these 14 viruses it seems more logical to consider them as strains of one virus group rather than

TABLE 2.—*Comparison of properties and host range of various crucifer viruses*

Virus	Properties [1]			Reaction on selected hosts [2]																		
	Dilution tolerance	Aging in vitro (hours at 20° C.)	Heating (°C. for 10 minutes)	Cabbage	Cauliflower	Broccoli	Kohlrabi	Kale	Brussels sprouts	Rape	Wallflower	White mustard	Black mustard	Turnip	Chinese cabbage	Dames violet	Annual stock	Tobacco	*Nicotiana glutinosa*	Cucumber	Currant tomato	Spinach	Swiss chard
Cabbage A (*16*)	1–10,000	192	60	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	S	O	O	S	S
Cabbage black ring (*15*)	1–1,000	72	59	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	S	S	O	S	O
Cabbage ring necrosis (*6*)	1–600	48	50	S		S	S	S	O	S	S	S	S	S	S	S	S	L	S	O	L	O	
Turnip mosaic (*3*)	1–100,000 [3]	72	54	S	S	O	O	O	O	O	O	O	O	S	S	S	S	L	S	O	O	O	
Turnip mosaic (*11*)	1–4,000	72	63	S		O			O	O	O	O	S	S	S	O	O	L	S			O	
Turnip mosaic (*2*)	1–1,000	72	60	S	O	S	O	O	O	O	O	O	S	S	S	O	O	L	S			O	
Turnip mosaic T_1 (*7*)	1–50,000	120	56	O	O	O	O	O	O	O	O	O	S	S	S	O	O	L	S			O	
Turnip mosaic T_6 (*7*)	1–50,000	84	56	O	O	O	O	O	O	O	O	O	S	S	S	O	O	L	S			O	
Turnip mosaic T_8 (*7*)	1–5,000	96	58	O	O	O	O	O	O	O	O	O	S	S	S	O	O	L	S			O	
Turnip mosaic T_9 (*7*)	1–50,000	72	58	O	O	O	O	O	O	O	O	O	S	S	S	O	O	L	S			O	
Severe stock mosaic (*12*)	1–4,000	192	60	O	O	O	O	O	O	O	O	O	S	S	S	O	O	L	S	O	O	O	
Mild stock mosaic (*12*)	1–5,000	144	65	O	O	O	O	O	O	O	O	O	S	S	S	O	O	L	S	O	O	O	
Rape mosaic (*8*)	1–7,000	384	68	S	S	S	S	S	S	S			S	S	S	O	O	L	S	O	O	O	O
Radish mosaic (*13*)	1–15,000	144	75	S	S	S	S	S	S	S	O	O	S	S	S	O	O	L	S	O	O	O	O
Cabbage B (*16*)	1–1,500	360	75	S	S	S	S	S	S	S			O	S	S	S	O	L	S	O	O	O	
Cauliflower mosaic (*10*)	1–3,000	192	80	S	S	S	S	S	S	S													
Broccoli mosaic (*1*)	1–3,000	96	75	S																			
Chinese cabbage mosaic (*14*)	1–6,000																						

[1] Values given are points at which inactivation occurred.
[2] S=Systemic infection; L=local reaction; C=no infection; no symbol=host not tested
[3] Inactivation was at 1–10,000 in 4 out of 5 trials.

as distinct viruses. The strain relationship of cabbage virus A and the black ring virus has been proved. These two viruses are considered as strains of turnip virus 1 (*3*) in view of the close similarities in symptoms, host range, and properties plus the fact that both virus A and turnip virus 1 were first obtained from the same area in southeastern Wisconsin. Turnip virus 1 is chosen as the type virus of the group because it was the first of the crucifer viruses for which critical property studies were made. Le Beau and Walker (*7*) have also considered turnip viruses T_1, T_6, T_8, and T_9 as strains of turnip virus 1. That the ring necrosis virus is a member of this group there is little doubt. It is the writers' opinion that the turnip viruses of Tompkins (*11*) and of Chamberlain (*2*), the two stock viruses, the rape mosaic virus (*8*), and the radish mosaic virus are also members of this group. The virus causing the ring spot disease of cabbage described by Smith (*9*) is very probably another strain of this group, but since no properties were given for this virus a close comparison with turnip virus 1 cannot be made.

Cabbage virus B, the cauliflower mosaic virus, the Chinese cabbage mosaic virus (*14*), and the broccoli mosaic virus (*1*) comprise a group that is quite distinct from the turnip virus 1 group in properties symptoms, and host range. These viruses are confined to the Cruciferae except for the local reactions of the Chinese cabbage virus on tobacco and *Nicotiana glutinosa*. Their characteristic symptoms are vein clearing and vein banding. The strain relationship of virus B and the cauliflower mosaic virus has been proved, and virus B is classed as a strain of cauliflower virus 1. In parallel inoculations to a few selected hosts in this laboratory the Chinese cabbage virus appeared to be quite similar to virus B and the cauliflower mosaic virus both in symptoms and in its tendency to become masked in cabbage at 28° C.

There appear, then, to be two groups of crucifer viruses, one group being represented by turnip virus 1 and the other group by cauliflower virus 1. This and other studies (*1*, *3*, *6*, *7*) indicate that members of the turnip virus 1 group are favored by relatively high temperatures, while those of the cauliflower virus 1 group are favored by relatively low temperatures. With each group, masking tends to occur at unfavorable temperatures. Strains within one group may show variation in their individual temperature reactions. For example, on some hosts virus A is distinctly more severe than the black ring virus at 28° and 16° C. On other hosts the two are practically indistinguishable at high temperatures, but at low temperatures their reactions are widely different, owing chiefly to the increased severity of the black ring symptoms. It is apparent, then, that the black ring virus is favored by lower temperatures than some other members of this group. Little differential temperature reaction has been observed among members of the cauliflower virus 1 group.

SUMMARY

When the reactions of cabbage virus A, cabbage black ring virus, cabbage virus B, and cauliflower mosaic virus on cabbage were studied at various temperatures, it was found that they fell into two distinct groups.

In the turnip virus 1 group, containing cabbage virus A and the black ring virus, the progress and severity of disease development varied directly with the air temperature to which the plants were exposed, symptoms (except for necrosis with the black ring virus) being most severe at 28° C. and mildest at 16°. The characteristic symptom of both viruses was a coarse chlorotic mottle accompanied by leaf malformation. However, some marked differences between the reactions of the two viruses to temperature were observed. Symptoms of virus A were distinctly more severe than those of the black ring virus at 28° and 24°, but at 20° and 16° the exact reverse was true.

In the cauliflower virus 1 group containing cabbage virus B and the cauliflower mosaic virus, symptom intensity was also found to be directly proportional to the air temperature. However, in contradistinction to the turnip virus 1 group, symptom intensity increased with decrease in temperature and complete masking occurred at 28° and 24°. The change in reaction with change in temperature was exactly parallel with both viruses. The characteristic symptoms of these viruses were chlorotic vein clearing and vein banding.

When either virus A or the black ring virus occurred in cabbage together with either virus B or the cauliflower mosaic virus, the resulting disease reaction was more severe than that produced by either virus alone. The increased severity of symptoms was so pronounced at 28° and 24° that they appeared as those of an entirely different disease. At low temperatures the activity of virus A or the black ring virus was so reduced that combination symptoms agreed very closely with those of virus B or of the cauliflower mosaic virus. In such combinations the black ring virus reacted very similarly to virus A, and the cauliflower mosaic virus reacted similarly to virus B. When the temperature at which plants infected with a virus combination were growing was reversed from high to low, or vice versa, there resulted a corresponding reversal in symptom type.

At high temperatures symptoms produced by virus A on *Brassica oleracea* var. *gemmifera*, *Nicotiana rustica*, and *N. multivalvis* were practically indistinguishable from those produced by the black ring virus, but at low temperatures the reactions of the two viruses on these hosts were markedly different. A striking effect of temperature on the type of symptoms produced by these two viruses on *N. glutinosa* is described.

By use of the differential reaction between virus A and the black ring virus on the hosts mentioned above, virus A was shown to effectively immunize cabbage against infection by the black ring virus. Likewise, the black ring virus protected cabbage plants against infection from virus A, as measured on *Solanum integrifolium*, a host which was completely differential for virus A. Both virus A and the black ring virus are classed as strains of *turnip virus* 1.

At both high and low temperatures the reaction of the cauliflower mosaic virus was much more severe than that of virus B on such hosts as *Brassica pekinensis*, *B. nigra*, *B. napus*, *B. arvensis*, and *B. campestris*. By the use of the differential reaction on *B. pekinensis*, virus B was shown to immunize cabbage against infection by the cauliflower mosaic virus. Virus B is classed as a strain of *cauliflower virus* 1.

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